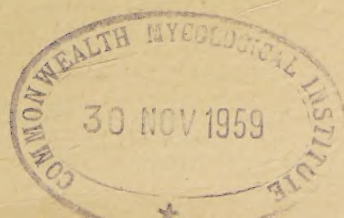


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THE APPLE LEAF-CURLING MIDGE, *Dasyneura mali* Kieffer, SEASONAL HISTORY, VARIETAL SUSCEPTIBILITY AND PARASITISM 1955-58

By D. H. TODD, Entomology Division, Department of Scientific and Industrial Research, Palmerston North

(Received for publication, 4 June 1959)

Summary

During the 1955-56 and 1956-57 seasons at Palmerston North, the apple leaf-curling midge, *Dasyneura mali* Kieffer, reached significant proportions. Infestation in 1957-58 was less than in the previous two seasons. There are normally five generations of the midge between early October and late April.

The parasite, *Proctogaster demades* Walker, attains a generally high level of parasitism of *D. mali*, but its failure to check the second generation restricts its effectiveness as a controlling agent. The use of suitable chemicals to control the second generation would complement the parasite during this period.

No apple varieties are entirely immune, and the degree of susceptibility and infestation is determined by the quantity of terminal growth present. No marked varietal preference was detected.

INTRODUCTION

The hymenopterous egg parasite, *Proctogaster demades* Walker, introduced to New Zealand in 1925 to control the pear leaf midge, *Dasyneura pyri* Bouché, was first reported by Todd (1956) to be attacking the apple leaf-curling midge, *Dasyneura mali* Kieffer, in this country.

The level of parasitism attained by the parasite during the 1954-55 season at Palmerston North was sufficiently high to justify further investigations being made to determine more fully its potentialities as a controlling agent of *D. mali*. The investigations were continued for a further three seasons, 1955-58, with particular attention directed to the level of parasitism and control being attained by *P. demades*. Seasonal history studies of the midge were also undertaken, and, during the 1956-57 season, trials were carried out to obtain information on susceptibility of some of the most commonly grown apple varieties to attack by the midge.

The investigations were again carried out in the orchard at Massey College, Palmerston North.

Dasyneura mali

SEASONAL HISTORY STUDIES

1955-56 Season

During this season, there were five generations of the midge between early October and the end of April. Only the early-leaving variety of apples were infested by the first generation adults, and for this reason the severity of attack at this stage was slight. During the pupal period of the first generation, a greater number of varieties commenced to produce terminal growth, and when the second generation adult females appeared, there was an abundance of oviposition sites, which resulted in a heavy infestation, and, in consequence, severe defoliation of the trees. The life span of the adult midge in the field is obviously short, this being particularly noticeable during the second generation. Maximum emergence of the adults of this generation extended over five days; three days after this period only a very limited number could be found. Overlapping occurred during subsequent generations, and for this reason they were not as clearly separated as the earlier ones. Throughout the remainder of the season, the various stages of the midge were present in the orchard at all times. However, by making regular observations, it was still relatively easy to determine the beginning and end of a generation. This was achieved by watching closely for the sudden and significant increase in the number of adult midges which normally signifies the beginning of a generation. An alteration in colour and appearance of infested foliage denotes the end of a generation. During early stages of infestation, the feeding larvae cause the leaves to become tightly rolled and red in colour. On completion of the feeding period the infested leaves are hard and brittle, and brown in colour. They ultimately become extremely ragged in appearance when the mature larvae evacuate them in search of pupation sites. A severe infestation by the third-generation adults again resulted in the defoliation of the varieties producing fresh terminal growth at that time. Owing to the overlapping, which was obviously occurring, and the fact that the severity of the third infestation was so great, little new growth was made by the trees before the fourth-generation adults emerged. In consequence, this generation was comparatively small, with only slight damage occurring. The fifth, and final, generation of the season was also small, the food supply being restricted not only by previous defoliation but also by influence of the season, as many of the trees had ceased to produce new foliage and practically all the new growth was confined to a limited number of watersprouts.

1956-57 Season

For the first part of the 1956-57 season, the cycles of the midge were comparable to those of the previous season, with adults from the overwintering generation emerging during the second week in October and infesting only the early-leaving varieties. The resultant attack was slight and did not affect a great number of trees. When the second generation adults emerged, a much larger number of trees were

carrying fresh terminal growth, with the result that the severity of the attack was considerably greater than that of the first generation. During this generation it became apparent that overlapping was occurring. However, it was still relatively easy to determine the period of maximum emergence and oviposition of the third-generation adults, which were present in extremely large numbers resulting in a heavy infestation and severe defoliation. No observations were made at the time the fourth-generation adults would have been emerging. Approximately a week after this assumed period, there was a lack of fresh terminal growth, a condition brought about, primarily, by the severity of the third-generation attack, and the abnormally dry season. It can be assumed that the fourth-generation adults did emerge under these conditions when there was a lack of oviposition sites, and an inadequate food supply for the larvae. This resulted in a considerable portion of the fourth generation being ineffective, progeny developing from comparatively few. Difficulty was experienced in following the seasonal history of the midge during the latter part of the season. However, with the earlier generations following a similar pattern to those recorded during the 1955-56 season, and the fact that the midge activities ceased in late April, it is reasonable to presume that there were five generations of the midge during the 1956-57 season.

1957-58 Season

During this season the cycles of the midge did not coincide with those of the two previous seasons. The period of maximum oviposition of the first generation adults was prolonged and did not cease until after mid October, and the two subsequent generations were appreciably later than those recorded for the 1955-56 and 1956-57 seasons. Abnormal weather conditions during the early part of the season were no doubt primarily responsible for this. Meteorological records kept by Grasslands Division, Palmerston North, show that below-average temperatures and strong winds were experienced during October 1957. Rainfall was also well above average with a total of 5.01 in. rain recorded for the month, 3.87 in. of which fell during the first 15 days. These conditions, and in particular the low temperatures, appear to have delayed the peak oviposition period of the new season's adults, and retarded development of the first generation. The effect of this upon subsequent generations was apparent. The average maximum and minimum and mean temperatures (screened) for October, years 1955-57, are:

Year	Average Maximum °F	Average Minimum °F	Mean °F
1955	64.5	46.6	56.6
1956	64.1	48.4	56.3
1957	60.9	44.8	52.9

The first generation of the season was estimated to be considerably smaller than that of corresponding generations of the two previous seasons. Of the very limited amount of terminal growth at the start of the season, only a small proportion became infested. Peak emergence of the second generation adults was of short duration, and

occurred when terminal growth was abundant. In spite of this, the degree of infestation was relatively slight with much of the susceptible foliage remaining clean and healthy. Much of this foliage, however, ultimately became infested by the small and fairly constant numbers of adults which persisted practically right through until the commencement of the third generation. This early overlapping appears to have resulted from the rather prolonged emergence and oviposition period of the first generation adults. With the bulk of the terminal growth already infested when the third generation adults emerged, egg laying was very concentrated on the few sites that were available. Throughout the remainder of the season, varying numbers of adults were constantly present, resulting in instant infestation of any new terminal growth or watersprouts. This situation prevented any further separation of generations, and in consequence the total number of generations for the season was not determined.

Details of the three years' seasonal history studies of *D. mali* are summarised in Table 1.

TABLE 1. SEASONAL HISTORY OF *D. mali* 1955-58 SEASONS

Generation	Period of Maximum Oviposition	Period of Maximum Hatching	Date Mature Larvae Commenced to Pupate	Date First Adults Emerged	Length of Cycle
1955-56 Season					
1st	8-12.10.55	13-15.10.55	8.11.55	28.11.55	51 days
2nd	28.11.55-2.12.55	2-6.12.55	23.12.55	3. 1.56	36 days
3rd	3-6.1.56	7-10.1.56	24. 1.56	10. 2.56	38 days
4th	10.2.56-†	†	27. 2.56	21. 3.56	40 days
5th	21-26.3.56	†	20. 4.56	Overwintering	—
1956-57 Season					
1st	10-14.10.56	16-20.10.56	14.11.56	27.11.56	48 days
2nd	27.11-2.12.56	2-7.12.56	†	6. 1.57	40 days
3rd	6-11.1.57	9-14.1.57	25. 1.57	*	*
4th	*	*	6. 3.57	*	*
5th	*	*	*	Overwintering	—
1957-58 Season					
1st	11-20.10.57	17-26.10.57	20.11.57	7.12.57	57 days
2nd	7-11.12.57	12-16.12.57	30.12.57	24. 1.58	48 days
3rd	24-30.1.58	29.1-4.2.58	14. 2.58	*	*
4th	*	*	*	*	*
5th	*	*	*	Overwintering	—

† No record—author absent owing to exigencies of other work.

* No data recorded owing to overlapping generations.

Prosactogaster demades

During the whole series of investigations, special attention was given to the level of parasitism and degree of control being attained by *P. demades*. Dissections of field-collected midge larvae were made to ascertain this. In order to make seasonal comparisons of the degree

of parasitism, it was decided to carry out the dissections during each generation of the midge just prior to the larvae becoming fully fed.

Parasitism 1955-56

A small number of *P. demades* adults were first observed on 11 October when the period of maximum oviposition by the first-generation adult midges was almost past. The numbers did increase during the following two weeks, but, owing to the scarcity of midge eggs, parasitism was relatively low. On 7 November, when 150 first-generation larvae were dissected, 44.7% parasitism was recorded, including 11 cases (16.4%) of multiple parasitism. During the following six weeks there was a noticeable absence of parasites, and with oviposition of the second-generation adult midges occurring during this period, it was evident that the level of parasitism would be extremely low. This proved to be the case, for when 500 second-generation larvae were dissected on 21 December, only 2.8% parasitism was recorded, including one case (7.1%) of multiple parasitism. By 23 December, parasite activity was definitely on the increase, and between 3 and 6 January, which was the period of maximum oviposition of the third-generation adult midges, large numbers of parasites were seen ovipositing in the eggs of their host. Of the 300 larvae dissected for parasitism on 26 January, 61.7% were parasitised, including 50 cases (27.0%) of multiple parasitism. From this stage on until *D. mali* became inactive at the end of April, there appeared to be a gradual build up of the parasite, the actual numbers at any one time fluctuating to some extent. The 300 fourth-generation larvae dissected on 2 March gave a figure of 89.7% parasitism, including 192 cases (71.4%) of multiple parasitism. Of the 114 fifth- and final-generation larvae dissected on 21 April, 95.6% were parasitised, including 90 cases (82.5%) of multiple parasitism. As many as 12 embryos were found in one host larva. The high level of multiple parasitism which prevailed during the final two generations of the midge can be attributed to host competition, brought about by a general decline in the incidence of the midge, mainly because of an insufficient food supply, and a build up of the parasite population.

Parasitism 1956-57

At the close of the 1955-56 season, some doubt existed concerning the chances of survival of the multiple-parasitised midge larvae during their over-wintering period. For full development of *P. demades*, it is necessary for its host to reach the cocoon stage (Todd 1956). Obviously, a high mortality of the larvae during the winter would adversely affect the numbers of adult parasites emerging at the commencement of the following season. However, this did not appear to be the case, for at the very onset of the 1956-57 season, large numbers of *P. demades* adults were observed on the wing, and heavy parasitism of the midge was recorded. Dissections of 250 first-generation midge larvae made on 6 November, gave a figure of 82.4% parasitism, including 130 cases (63.2%) of multiple parasitism. This high level of multiple parasitism was again created by the low incidence of the midge, resulting in strong competition for hosts. Over the period of maximum oviposition of the second-generation adult midges during

the final week in November and early December, there was almost a complete absence of the parasite. In consequence, an extremely low figure of 0.7% parasitism was recorded when 300 second-generation larvae were dissected on 18 December. No multiple parasitism was recorded. As was the case during the previous season towards the end of December, a gradual increase in the number of *P. demades* adults was observed, and between 6 and 11 January, when the period of maximum emergence of the third-generation adult midges occurred, a large number of parasites were present. Three hundred third-generation larvae were dissected on 24 January, when 78.7% parasitism was recorded, including 90 cases (38.1%) of multiple parasitism. Owing to overlapping generations of the host midge for the remainder of the season, it was not possible to follow any further the cycle of *P. demades*. However, with the presence of a large number of parasites during the latter part of the season, it was quite apparent that heavy parasitism of the midge was occurring. At the end of the season, when 150 larvae were dissected on 4 April, 113 were found to be parasitised, giving a level of parasitism of 75.3%, including many cases of multiple parasitism.

Parasitism 1957-58

During this season the behaviour of the parasite, *P. demades*, was in general similar to that recorded for previous seasons. Adults were fairly numerous and active when maximum oviposition by first-generation midges occurred. The numbers gradually decreased however, and, by the end of October, none could be found. Of the 200 first-generation larvae dissected for parasitism on 12 November, 60.5% were parasitised, with 29 cases (23.9%) of multiple parasitism. There was no significant increase in the number of parasites until the third week in January when third-generation adult midges were estimated to be at their maximum. During the period between the first and third generations there was little parasite activity. As a result, parasitism was at a low level for the second generation. Of the 300 second-generation larvae dissected for parasitism on 3 January, only 1.7% were parasitised. The presence of the parasite when the third generation adult midges were ovipositing gave some assurance of a reasonable level of parasitism. For some inexplicable reason however, this was not as high as had been expected, and significantly lower than that recorded for corresponding generations of previous seasons. Of the 300 larvae dissected on 17 February, 47.3% were parasitised, with 18 cases (12.7%) of multiple parasitism. No further records of parasitism were taken, but all subsequent examinations during the remainder of the season revealed the presence of varying numbers of parasites.

Details of parasitism over three years are summarised in Table 2.

VARIETAL SUSCEPTIBILITY

Trials 1956-57

Twelve of the most commonly grown apple varieties were chosen for the investigations. These were: Gravenstein, Cox's Orange, Kidd's

TABLE 2. PERCENTAGE PARASITISM BY *P. demades* 1955-58 SEASONS

Generation	Date Larvae Dissected for Parasitism	No. Dissected	Total % Parasitism	% Multiple Parasitism
1955-56 Season				
1st	7.11.55	150	44.7	16.4
2nd	21.12.55	500	2.8	7.1
3rd	26. 1.56	300	61.7	27.0
4th	2. 3.56	300	89.7	71.4
5th	21. 4.56	114	95.6	82.5
* 1956-57 Season				
1st	6.11.56	250	82.4	63.2
2nd	18.12.56	300	0.7	0.0
3rd	24. 1.57	300	78.7	38.1
4th	4. 4.57	150	75.3	†
5th				
* 1957-58 Season				
1st	12.11.57	200	60.5	23.9
2nd	3. 1.58	300	1.7	0.0
3rd	17. 2.58	300	47.3	12.7

* Owing to the impossibility of separating the fourth and fifth generations of the midge during the 1956-57 season, no parasitism figures are given for these two separately. For similar reasons, parasitism figures for the 1957-58 season are restricted to the first three generations.

† % multiple parasitism not recorded, but many cases occurred.

Orange (Red), Jonathan, Delicious, Delicious (Golden), Sturmer, Granny Smith, Rome Beauty, Statesman, Dougherty, Ballarat.

Ten-year-old trees, growing on East Malling IX apple stocks, in rows five feet apart with approximately equal exposure to infestation, were selected. Two trees of each variety were examined, these being separated by four feet within the rows.

In an attempt to determine the susceptibility of the varieties chosen, the percentage of infested tips was recorded, population levels were compared, and the severity of attack was estimated (Table 3). It had been proposed to collect these data for each generation of the midge, but this was not possible owing to the absence of growth suitable for oviposition on a large proportion of the varieties under trial, particularly at the beginning and end of the season.

To determine the percentage of infested tips occurring on any one variety, the terminal growth on each of the two trees of each variety was examined for midge eggs. This procedure was carried out during the periods of maximum oviposition of the midge, and, in each case, twenty tips selected at random were examined, ten from each tree.

The population levels were compared by dissecting five random-collected infested leaves of comparable size from each of the varieties under trial. The larvae from each leaf were counted and the population level recorded as the mean number of larvae per leaf.

TABLE 3. SUSCEPTIBILITY TRIALS, 1956-57

Apple Variety	1st Generation Mean Larval Population per Infested Leaf	2nd Generation			3rd Generation		
		Degree of Infestation		Severity of Attack	Degree of Infestation		Severity of Attack
		No. Tips Examined	% Infested		No. Tips Examined	% Infested	
Gravenstein	17.2	20	65.0	Severe	20	10.0	Slight
Cox's Orange	*	20	15.0	Nil	20	100.0	Moderate
Kidd's Orange (Red)	*	20	35.0	Moderate	20	10.0	Slight
Jonathan	*	20	0.0	Nil	20	20.0	Nil
Delicious	*	20	70.0	Severe	20	35.0	Moderate
Delicious (Golden)	*	20	30.0	Moderate	20	35.0	Slight
Sturmer	*	20	15.0	Nil	20	10.0	Nil
Granny Smith	*	20	70.0	Moderate	20	100.0	Severe
Rome Beauty	*	20	0.0	Nil	20	15.0	Nil
Dougherty	46.8	20	100.0	Severe	20	10.0	Nil
Statesman	*	20	70.0	Severe	20	5.0	Nil
Ballarat	28.0	20	100.0	Severe	20	0.0	Nil

* Varieties not infested owing to absence of terminal growth.

† No record—mature larvae had dropped to ground before leaves could be dissected.

The severity of attack on the selected varieties was estimated by visual observations at the periods when the infested leaves were in the typical curled condition, and showing red colour. In each case the attack was recorded as being:

- Nil — curled leaves present, but too few to be of any consequence.
- Slight — small number of curled leaves present.
- Moderate — conspicuous number of curled leaves present.
- Severe — large number of curled leaves present.

When the midge activities commenced in early October, only three of the chosen varieties, Gravenstein, Dougherty, and Ballarat had produced new foliage. The results of larval counts on these varieties are shown in Table 3. At the commencement of the second generation of the midge in early December, all the trial varieties were carrying new terminal growth, thus allowing the proposed trial programme to be carried out to the full. Similar conditions prevailed for the third generation which commenced in early January, but, unfortunately, in this instance it was not possible to record the population levels for all the varieties, because in some cases fully fed larvae dropped to the ground before leaves could be collected for dissection. Owing to the absence of new foliage on the trial trees over the remainder of the season, no further observations were made.

DISCUSSION

The seasonal history studies of *D. mali* carried out over the past three years indicate fairly clearly that the severest infestations occur during the second and third generation of the midge, at a time when most trees are making maximum growth. Only the early-leaving varieties are attacked by the first spring emergence. The fourth and fifth generations are relatively small, mainly owing to an inadequate food supply caused by previous defoliation and the inability of most trees to produce terminal growth at the latter part of the season. Rainfall appears to have a definite effect on the degree of infestation by influencing the amount of new growth. The degree of midge damage occurring in an orchard appears to be determined by two factors. The most important of these is considered to be the availability of oviposition sites for the female midges, and consequent adequacy of food for the feeding larvae. The second factor appears to be the failure of the parasite to check the second generation of the midge.

The information obtained from the susceptibility trials indicates conclusively that none of the apple varieties under trial was entirely immune from attack. It was not possible to detect any marked preference among the different varieties, but there are factors which do have a definite bearing on susceptibility and degree of infestation. The most prominent of these is the presence or absence of terminal growth on the tree when adult midges are on the wing, particularly during the period of maximum emergence and oviposition. This point is well illustrated in Table 3, first generation, where only the varieties carrying new seasons growth are attacked. In many instances the terminal growth on a particular variety is confined to a small proportion of

the branches on a tree, a factor which would obviously influence the degree of infestation. There appears to be little or no relationship between the larval population per leaf and susceptibility. Generally, the largest numbers occur when there has been strong competition for oviposition sites by the female midges, resulting in a concentration of eggs on the available leaves.

In spite of the generally high level of parasitism obtained by *P. demades*, the apple leaf-curling midge, *D. mali* still continues to reach significant proportions, particularly during December and January when the second and third generations of the midge occur. Records kept over the past three years show that the life cycle of *P. demades* is not as well synchronised with that of its host as was first thought (cf. Todd 1956). It is present in reasonable numbers at the beginning and during the latter part of the season, but is almost entirely absent when the second generation of the midge occurs. During this period most trees are making maximum growth and are particularly vulnerable to attack, resulting in a significant build up of *D. mali*. In consequence, the incidence of the midge during the third generation is extremely high, a situation which the parasite cannot adequately deal with in spite of its efficiency at this stage. A progressive build-up of *P. demades* over the latter part of the season, and a general decline in the incidence of the midge, because of an insufficient food supply, results in heavy parasitism of both the fourth and fifth generations. The relatively high level of multiple parasitism which prevails at this stage can be attributed to strong competition for hosts.

The almost complete absence of the parasite during the second generation of the midge shows clearly that the parasite generations are not synchronised with those of its host. The first and second generations of the midge are fairly clear cut with very little or no overlapping between them. It would seem that while the parasite is present during the first generation of the midge, there is no parasite generation to coincide with that of the second midge generation; for example it is possible that it emerges after the midge. From the second midge generation onwards however, there is much overlapping and midges in all stages can be found at all times, thus ensuring that the parasite can find midge eggs for parasitisation whether or not the parasite generations coincide with those of the midge. Under such conditions it is not surprising that parasitism builds up after the second generation of the midge even though the parasite is absent during this generation.

From the control aspect, the most important feature arising from the investigations is the knowledge that there may be now a distinct possibility of achieving a much higher degree of control of the midge than in the past. The knowledge now available on the seasonal cycles of both *D. mali* and its parasite, *P. demades*, should enable future chemical control programmes against the pest to be planned with a greater degree of accuracy than has been previously possible, and at the specific time of the season when the parasite is practically ineffective. Although the life history of *D. mali* has been worked out satisfactorily at Palmerston North, on account of varying climatic conditions and seasonal trends it may not be synchronous throughout

its range, and there could also be variations from one season to another. A close study of these factors would be of utmost importance if chemical control is contemplated, and if maximum effect is to be achieved.

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PLANT PATHOGENICITY STUDIES WITH NON-SPORING FUNGI FROM TUSSOCK-GRASSLAND SOILS

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(Received for publication, 5 May 1959)

Summary

Some strains of an unnamed *Rhizoctonia* sp., *Rhizoctonia solani*, and a Sterile Mycelium, isolated from tussock grassland soils, show no pathogenic activity against lettuce, radish, wheat, potatoes, or pines. The fungi do not form mycorrhizal associations with *Pinus radiata*.

INTRODUCTION

Species of *Rhizoctonia* are frequently encountered as pathogens of many plants. *Rhizoctonia* or similar sterile mycelia are also frequently isolated from many root systems and are capable of forming some kind of association with root tissues (Harley 1950). In tussock-grassland soils which have been examined in this laboratory, *Rhizoctonia* spp. and sterile mycelia have been found to be the predominant fungi (Thornton 1958). It is not known whether they occur there solely as saprophytes or in part, at least, as plant parasites. As a first step in elucidating this question, experiments were made to determine whether these fungi have any pathogenic properties for some of the commonly employed test plants. Their ability to form mycorrhizal associations with *Pinus radiata* has also been investigated.

FUNGI

The present strains of fungi were isolated from tussock-grassland soils at Waiouru and Alexandra. Identification of the unnamed *Rhizoctonia* sp. and *Rhizoctonia solani* was kindly provided by Dr. A. Kerr, Waite Agricultural Institute. These cultures have been deposited in the herbarium of the Commonwealth Mycological Institute and have been assigned the following I.M.I. herbarium numbers: Sterile Mycelium 1T, 71568; *Rhizoctonia* sp. 3T; *Rhizoctonia* sp. 4T, 71570; *Rhizoctonia solani*† 6T, 71572. Sterile Mycelium 1T has been described by Thornton (1958) as Fungus S.B. 235.

METHODS AND RESULTS

All experiments were conducted in an unheated glasshouse at the Taita Experimental Station, Lower Hutt.

A separate pot, or other vessel, was used for each kind of test plant and for each strain of fungus in this study.

* Present address: Botany Department, University of Otago, Dunedin.

† This culture has been disposed in the herbarium of the Commonwealth Mycological Institute as *Corticium solani* (Prill. et Delacr.) Bowd. et Golz.

Effect of Fungi on Lettuce, Radish, Wheat, and Potatoes in Taupo Soil

Cultures of *Rhizoctonia* sp. 3T, and Sterile Mycelium 1T were grown at room temperature (*ca.* 7–18°C) for 35 days in sterilised moistened corn meal. Clay pots (23 cm diameter $\times$ 26 cm deep) were filled with Taupo hill soil, collected at a depth of 0–6 in. under tussock grassland at Waiouru, and sterilised at 20 lb/in.² for 3 hours. A culture of the fungus on corn meal (*ca.* 40 g) was mixed with the top 10 cm of soil in each pot. Seeds of lettuce, radish, and wheat, and potato tubers, were surface-sterilised with an aqueous solution of mercuric chloride (0.1% w./v.) for 1–2 minutes and washed 8–10 times with distilled water. Two potatoes were sown in soil inoculated with Sterile Mycelium 1T, and the other seeds (4–6 pairs) in pots of soil inoculated with each fungus. Pots of uninoculated soil were also sown with seeds and served as controls. All pots were watered regularly with distilled water.

Growth of plants was poor in all treatments, including the controls, probably because of mineral deficiencies in this infertile soil, but their development was normal and no evidence of fungal infection with *Rhizoctonia* sp. 3T or Sterile Mycelium 1T was seen. However, some infection with a species of *Rhizoctonia* occurred on a potato plant in the control series. After growth for 6–8 weeks, an examination of fungi in the soil was made by a modification of the Rossi-Cholodny technique (Blair 1945) using 2–4 slides for each pot. Fungi possessing the characteristic morphology of *Rhizoctonia* sp. 3T or Sterile Mycelium 1T were detected in all inoculated soils. These fungi were not detected in the control soils supporting growth of lettuce, wheat, or potatoes, but a species of *Rhizoctonia* was present on half of the Rossi-Cholodny slides from the control soil supporting growth of radishes.

These results indicate that the *Rhizoctonia* sp. 3T and Sterile Mycelium 1T are not pathogenic for these plants under the experimental conditions employed.

Effect of Fungi on Potatoes in Perlite and Vermiculite

In view of the possibility that the soil in the above experiments had been incompletely sterilised, the test with potatoes was repeated with a mixture of expanded perlite and vermiculite as medium. Cultures of *Rhizoctonia* sp. 3T, *Rhizoctonia solani* 6T and Sterile Mycelium 1T were grown for 56 days in this medium moistened with Richard's peptone medium, with glucose, however, omitted. Approximately 40 ml of these inocula were mixed with the top 10 cm of the sterilised perlite and vermiculite medium contained in glazed pots (23 cm diameter $\times$ 29 cm deep). Potatoes were surface-sterilised with aqueous mercuric chloride as before and sown in these pots which were regularly watered and fertilised with the mixture of inorganic salts described by Morrison (1956). The potatoes grew normally for 8 weeks with no evidence of infection by the fungi.

After growth of the above potato plants for 8 weeks, developing tubers were exposed in the pots and incisions made in them with a sterile needle. Fresh inocula of the test fungi (6 mm discs from

growth on Czapek-Dox agar) were placed against these incisions and, after replacing the soil, plants were allowed to grow for a further 6 weeks. Hyphae of all species penetrated the incisions and covered the surrounding surface of the tubers. However, no parasitic infection occurred.

Dr. A. Kerr (pers. comm.) has also found the present strains to be non-pathogenic on a range of host plants.

Effect of Fungi on Plants of Pinus radiata

Jacks (1956) has pointed out that *Pellicularia* (*Rhizoctonia*) is one of the principal causal agents of damping-off of pine seed. Accordingly tests were made with the fungi used in the present study.

- (a) Non-mycorrhizal pines (*Pinus radiata*) were grown from seed in a sterilised perlite-humus mixture and four seedlings were transplanted to open pots of the same medium which had been inoculated with one of the four fungi under test. The pines did not show signs of damping-off or other infection after growth for 6 weeks.
- (b) Seeds of *Pinus radiata* were surface-sterilised with an aqueous filtered solution of bleaching powder (6.6% w./v.) for 60 minutes and sown, without washing, in petri dishes containing moist, sterilised perlite. Seedlings were transferred singly, under aseptic conditions, to plugged sterilised separating funnels (500 ml or 1000 ml) containing sterilised perlite and stones for drainage. Duplicate flasks were inoculated with a culture of the fungus, grown, as before, in the perlite medium. The medium was saturated with the following nutrient solution: $\text{NH}_4\text{H}_2\text{PO}_4$, 0.29 g; KNO_3 , 1.51 g; $\text{Ca}(\text{NO}_3)_2$, 1.64 g; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 1.23 g; Fe-HEEDTA (N-hydroxy-ethyl-ethylene-diamine-triacetic acid), 0.05 g; H_3BO_3 , 0.03 g; $\text{MnSO}_4 \cdot 4\text{H}_2\text{O}$, 0.025 g; $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$, 0.0001 g; $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$, 0.0003 g; $\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$, 0.0002 g; malt extract, 0.25 g; water, 5 l. Control flasks were not inoculated with fungi.

Although flasks received no further water or nutrients, growth of both fungi and pines was good. One such flask maintained healthy but limited growth of a pine seedling for 28 months. No damping-off of pine seedlings occurred. Pines were examined when about 10–12 cm high, but were found to have formed no mycorrhizal associations with the *Rhizoctonia* spp. or Sterile Mycelium.

ACKNOWLEDGEMENTS

We wish to thank Dr. A. Kerr, Waite Agricultural Research Institute, University of Adelaide, Australia, for identifying these fungi and for a report of their pathogenicity.

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THE INSECT COMPLEX OF SOWN PASTURES IN THE NORTH ISLAND

V. THE HYMENOPTERA AS REVEALED BY SUMMER SWEEP-SAMPLING

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Summary

A sweep-sampling survey during the summer of 1957 revealed 90 species of Hymenoptera in the main pasture types occurring in the North Island. The identity, distribution, and abundance of these species are indicated, and their possible economic importance is discussed. Thirty-one genera are new records for New Zealand.

INTRODUCTION

During the summer of 1957, a survey of insects associated with pastures in the North Island was carried out. In an earlier paper (Cumber 1958) techniques, coverage, and relevant sampling-station details are given, and the insect complex generally as shown by sweep-sampling is analysed and discussed. The information in respect of the Hemiptera (Cumber 1959a), Diptera (Cumber and Harrison 1959) and Coleoptera (Cumber 1959b) has already been indicated.

In the present account the Hymenoptera which were taken are discussed with regard to the identity of the species, their distribution, numbers encountered, and possible importance in the light of their systematic position and known habits.

The survey involves 113 paddock and 108 adjacent roadside samples each comprising a single sweep from an area of approximately 8 square yards.

Distribution maps for the 24 more commonly encountered species are presented, the presence of a species being indicated by a black dot in contrast to a ring where sampling failed to reveal its presence.

In the account which follows an asterisk is used to indicate a genus or species not previously recorded for New Zealand.

RESULTS

ICHNEUMONOIDEA

Fam. ICHNEUMONIDAE (Ichneumon Flies)

The great majority of insects belonging to this family are parasites of the larval and pupal stages of Lepidoptera, although some are known to parasitise Coleoptera, Diptera, Hemiptera, Hymenoptera, and Neuroptera. They include a great number of highly beneficial species.

Hy. 36A *Cremastus* sp.*

This species (3 females, 5 males) was taken in paddock samples from Taupo-Rotorua 4.3.57 (1 specimen) and Manaia (Taranaki) 3.4.57 (2), and in roadside samples from Wairoa 12.2.57 (1), Mangaweka 18.3.57 (3), and Eketahuna-Pahiatua 25.3.57 (1). Host relationships are not known.

Hy. 36 *Horogenes* sp.*

This species (6 females, 5 males) was taken in paddock samples from Opotiki 14.2.57 (3 specimens), Opotiki-Whakatane 14.2.57 (3), Hikateruru 6.3.57 (1), and Marton 27.3.57 (2), and in roadside samples from Mokau 24.1.57 (1), and Whakatane 14.2.57 (2). Host relationships are not known.

Hy. 24 *Ichneumon promissorius* Erich.*Distribution*

This species occurs in Australia and throughout New Zealand. In the present survey single males were taken at Helensville 23.1.57, Tutira 11.2.57, and Palmerston North (Pahiatua Track) 25.3.57.

Habits and Economic Significance

Parrott (1953a) states "The hosts of *I. promissorius* are usually noctuid larvae and have been reared from a number of different species in Australia and must be considered a valuable agent in the natural control of army worms and cut-worms."

Hy. 10 *Lissopimpla excelsa* (Costa)*Distribution*

Tillyard (1926) states that this species is "found in both countries (probably introduced into New Zealand)." It appears to be of widespread occurrence in both Islands. A single specimen was taken in a paddock sample from Wairoa on 12.2.57.

Habits and Economic Significance

Gourlay (1930) records the following hosts: *Oxycanus* (*Porina*) *umbraculata* Guen. O. (*P.*) *cervinata* Walk., *Aletia unipuncta* Haw. (*Pseudaletia separata* (Walk.)), and *Orthoclydon praelectata* Walk. This species probably plays a part in reducing the populations of subterranean caterpillars and possibly noctuid larvae.

Hy. 64 *Limnerium muelleri* Butler

A single specimen was taken in a roadside sample from Te Kuiti on 24.1.57. According to Gourlay (1928) this species is parasitic on *Morova subfasciata* Walk. (Thyrididae) which produces galls on *Muehlenbeckia australis* Meissn.

Fam. BRACONIDAE

The majority of species in this family are beneficial insects and, as in the preceding family, parasitise mainly the caterpillars of Lepidoptera. Some species, however, are parasites of aphids, scale insects, and Coleoptera,

Hy. 9 *Apanteles ruficrus* (Hal.)*Distribution*

This is a cosmopolitan species. In the present survey it was taken in 14 paddock (22 specimens) and 9 roadside (12) samples (16 females, 18 males). It is of widespread occurrence in the North Island (Fig. 1).

Habits and Economic Significance

Apanteles spp., because of their importance as biological control agents, have been studied by many workers. Parrott (1954), in reference to *A. ruficrus*, states "It appears to be common and widely distributed throughout New Zealand. It has been recorded as a parasite of *Plusia chalcites* Esp. and a species of *Tortrix*." In his Australian list, Parrott (1953b) records the following noctuid hosts: *Agrotis* sp., *Heliothis armigera* Hubn., *Leucania unipuncta* Haw. (*Pseudaletia separata* (Walk.)), and *Plusia* sp. In the present survey it was taken in January (5 samples), February (13), and March (5). This species is probably an important control agent of cutworms, army worms, etc.

Hy. 9A ? *Apanteles* sp.

A single female was taken in a roadside sample from Norsewood on 26.3.57.



Fig. 1



Fig. 2

Fig. 1.—Distribution map for the braconid *Apanteles ruficrus*. Hy. 9.

Fig. 2.—Distribution map for the braconid *Aspilota* sp. Hy. 22.

Hy. 22 *Aspilota* sp.*

This species (17 females, 64 males) was taken in 26 paddock (72 specimens) and 11 roadside (19) samples, the greatest catch (16) being in a paddock sample from Alton taken on 24.1.57. This species is widely distributed in the North Island (Fig. 2). Host relationships are not known. It was taken in January (8 samples), February (9), March (19), and early April (1).

Hy. 30 *Chorebus* sp.*

This species (3 females, 6 males) was taken in 5 paddock samples from Victoria Valley (Kaitaia) 16.1.57 (1 specimen), Waitoa 5.3.57 (1), Morrinsville 5.3.57 (1), Hunterville 26.3.57 (2), and Marton 27.3.57 (4). Host relationships are not known.

Hy. 29 *Macrocentrus* ? *rubromaculatus* (Cam.)*Distribution*

The genus is present both in Australia and New Zealand. The species (34 females, 28 males) was taken in 18 paddock (42 specimens) and 13 roadside (20) samples, the greatest catch (7) being in a paddock sample from Morrinsville taken on 5.3.57. It is widely distributed in the North Island (Fig. 3).

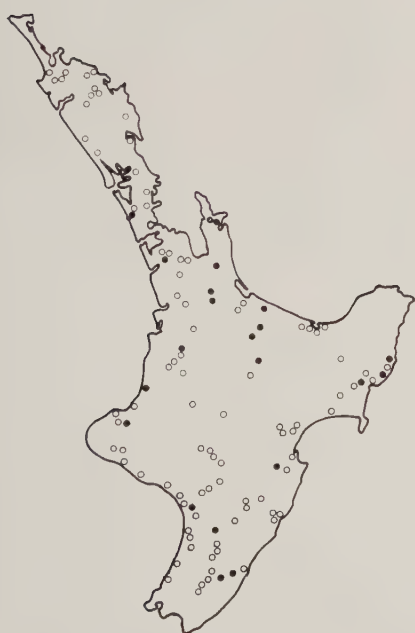


Fig. 3

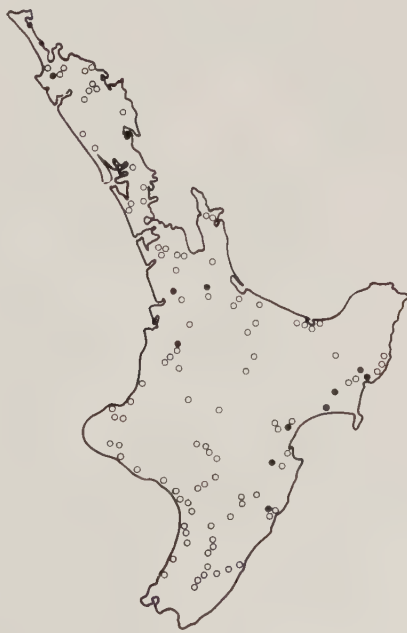


Fig. 4

Fig. 3.—Distribution map for the braconid *Macrocentrus* ? *rubromaculatus*. Hy. 29.

Fig. 4.—Distribution map for the braconid *Microplitis* sp. Hy. 14.

Habits and Economic Significance

Gourlay (1930) records *Melanchra* sp. as a host. Parrott (1953b, 1954) indicates in addition that *M. rubromaculatus* is parasitic on *Persectania aversa* (Walk.), *P. steropastis* (Meyr.), *Agrotis* sp., and allied noctuids. In the present survey it was taken in January (3 samples), February (7), March (20), and early April (1). This species probably plays an important part in controlling army-worm and cutworm outbreaks.

Hy. 14 *Microplitis* sp.*

This species (3 females, 13 males) was taken in 5 paddock (5 specimens) and 7 roadside (11) samples. It is of widespread occurrence in the North Island (Fig. 4). Host relationships are not known. It was taken in January (2 samples), February (6), and March (4).

Hy. 39 *Opius* sp.*

Some 10 species are recorded in Australia. It was taken (16 females, 24 males) in 15 paddock (34 specimens) and 5 roadside (6) samples, the greatest catch (8) being in a paddock sample taken at Mokau on 24.1.57. It is of widespread occurrence in the North Island (Fig. 5). The Australian representatives are commonly parasitic on trypetid flies. R. D. Eady (pers. comm.) indicates that "This is probably from an agromyzid such as the daisy miner." It was taken in January (15 samples), February (4), and March (1).

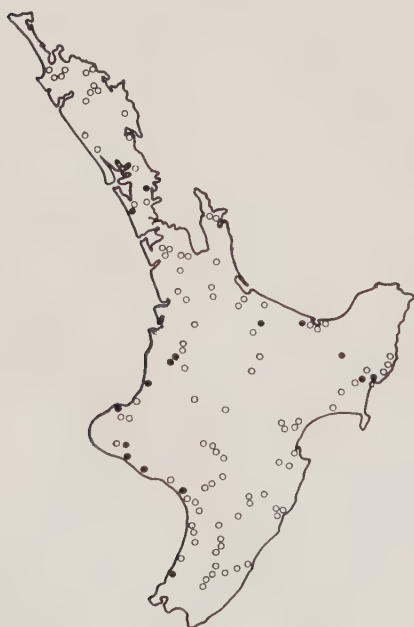


Fig. 5



Fig. 6

Fig. 5.—Distribution map for the braconid *Opius* sp. Hy. 39.

Fig. 6.—Distribution map for the braconid *Trioxys* sp. Hy. 20.

Hy. 61 *Rogas* spp.

Rogas spp. (3 females, 9 males) were taken in paddock samples from Gisborne-Tiniroto 12.2.57 (2 specimens), Tolaga Bay 12.2.57 (1), Waikohu 13.2.57 (1), Rangipo 4.3.57 (1), Taumarunui 8.3.57 (1), Ohakune 18.3.57 (1), and Eketahuna-Pahiatua 25.3.57 (1), and in roadside samples from Takapau 11.2.57 (1), Gisborne-Tiniroto 12.2.57 (2), and Ohakune 18.3.57 (1), indicating either a southern distribution in the North Island, or more likely, a relatively late-appearing species. Two species may be represented here.

The genus, which is of world-wide distribution, has been recorded in New Zealand as a parasite of *Orthoclydon praelectata* Walk. (Gourlay 1930). The writer has noted parasitism of early-stage noctuid larvae which is typical of *Rogas* attack, and in this capacity the species may be important in the control of army worms and cutworms.

Hy. 20 *Trioxys* sp.*

This species (15 females, 40 males) was taken in 19 paddock (53 specimens) and 2 roadside (2) samples, the greatest catch (13) being in a paddock sample from Helensville on 23.1.57. It is widely distributed in the North Island (Fig. 6). Host relationships are not known. It was taken in January (9 samples), February (6), and March (6).

Hy. 44. Undetermined†

A single female was taken in a paddock sample from Marton on 27.3.57.

CYNIPOIDEA

Fam. CYNIPIDAE

Included in this family are the Cynipinae which are gall formers orinquilines in galls, the Charipinae which are mainly hyperparasites of aphids or coccids, and the Eucilinae many of which parasitise Diptera. Tillyard (1926) records introduced cynipid species in New Zealand. Gourlay (1930) records *Allotria brassicae* Ashm. as a hyperparasite of *Aphis brassicae* (L.).

Hy. 17A *Charips* sp.*

A single female was taken in a roadside sample from Eketahuna-Pahiatua on 25.3.57.

Hy. 17 *Kleidotoma* spp.*

The genus is of world-wide distribution. Apparently several species are concerned in the present instance. They (9 females, 26 males) were taken in 11 paddock (15 specimens) and 13 roadside (20) samples. The genus is widely distributed in the North Island (Fig. 7). According to J. Quinlan (pers. comm.) these species are parasitic on small Diptera. In this capacity they are probably beneficial.

† Undetermined species stand in the collection of the Entomology Division, Palmerston North, under the numbers shown.

Fam. FIGITIDAE

The family includes parasites of Diptera and Neuroptera.

Hy. 52 *Anacharis* sp. ? *zelandica* Ashm.

Tillyard (1926) records one figitid species *A. zelandica* from New Zealand. J. Quinlan (pers. comm.) indicates that the species submitted is a parasite of Hemerobiidae and Chrysopidae. The writer has taken the species in association with the hemerobiid *Micromus tasmaniae* Walk. on several occasions so that it is a suspected parasite of this species.

In the present survey single specimens were taken in paddock samples from Dargaville 22.1.57, Warkworth 22.1.57, and Mapiu 8.3.57, and in roadside samples from Waverley 24.1.57 and Whakatane 14.2.57.



Fig. 7



Fig. 8

Fig. 7.—Distribution map for the cynipids *Kleidotoma* spp. Hy. 17.

Fig. 8.—Distribution map for the pteromalid near *Eupteromalus* sp. Hy. 5.

CHALCIDOIDEA

Fam. EURYTOMIDAE

The members of this family exhibit widely diverse habits. Some are egg parasites of orthopterous insects, others are associated with the nests of bees and wasps. Many species are gall formers or associated with galls formed by Diptera and Hymenoptera. Others attack seeds especially of leguminous plants and in this capacity are of considerable economic importance.

Hy. 11 Undetermined

A single specimen was taken in a paddock sample from Gisborne-Tiniroto on 12.2.57.

Hy. 77 Undetermined

A single specimen was taken in a roadside sample from Taupo-Rotorua on 4.3.57.

Fam. PTEROMALIDAE

Imms (1957) states that "This family is the largest among chalcids and its members, like those of the Encyrtidae, affect almost all orders of insects either as parasites or hyperparasites."

Hy. 5 Gen. near *Eupteromalus**

This species (411 females, 57 males) was taken in 74 paddock (339 specimens) and 50 roadside (129) samples, the greatest catch (28) being in a paddock sample from Takapau taken on 11.2.57. The species is widely distributed in the North Island (Fig. 8). Host relationships are not known. It was taken in January (36 samples), February (35), March (50), and early April (3).



Fig. 9

Fig. 9.—Distribution map for the pteromalid near *Eupteromalus* sp. Hy. 5A.



Fig. 10

Fig. 10.—Distribution map for the pteromalid near *Eupteromalus* sp. Hy. 5B.

Hy. 5A Gen. near *Eupteromalus**

This species (171 females, 54 males) was taken in 62 paddock (148 specimens) and 39 roadside (77) samples, the greatest catch (8) being in a paddock sample from Takapau taken on 11.2.57. This species is widely distributed in the North Island (Fig. 9). Host relationships are not known. It was taken in January (29 samples), February (27), March (41), and early April (4).

Hy. 5B Gen. near *Eupteromalus**

This species (all females) was taken in 48 paddock (189 specimens) and 25 roadside (59) samples, the greatest catch (20) being in a paddock sample from Takapau taken on 11.2.57. It is widely distributed in the North Island (Fig. 10). Host relationships are not known. It was taken in January (28 samples), February (28), and March (17).

The species 5, 5A, and 5B according to M. W. R. de V. Graham (pers. comm.) belong "very likely to an undescribed genus." It is suspected that the separate species recognised in the present account may be variants of a single species. The almost universal appearance of these species suggests that they may be parasites of Diptera.

Hy. 76 *Notoglyptus* sp.*

This species (all females) was taken in paddock samples from Alton 24.1.57 (1 specimen), Taihape 18.3.57 (1), and Taupo-Rotorua 4.3.57 (4), and in a roadside sample from the last named (3).

Hy. 63 *Ophelosia* sp.

Females were taken in a roadside sample from Pukekohe 23.1.57 (1 specimen), and in paddock (1) and roadside (2) samples from Otaki on 31.1.57. Gourlay (1930) records *Ophelosia dactylopii* Timb. as parasitic on *Pseudococcus* sp. in New Zealand and Australia.

Hy. 23 *Trichomalus* sp.*

This species (all females) was taken in paddock samples from Piopio 24.1.57 (1 specimen), Hihitahi 18.3.57 (2), and Marton 27.3.57 (2), and in roadside samples from Mohaka River (Napier-Taupo) 15.2.57 (1), Hunterville, 18.3.57 (1), Masterton 25.3.57 (1), and Kohinui 28.3.57 (1). Host relationships are not known.

Hy. 4 Undetermined

This species (all females) could not be placed in a known genus. It was taken in paddock samples only from Himatangi 30.1.57 (2 specimens), Otaki 31.1.57 (1), Levin 31.1.57 (1), Te Kuiti 8.3.57 (1), and Tarata 2.4.57 (1).

Hy. 49 Undetermined

Single females were taken in paddock samples from Ruakaka 21.1.57, and Himatangi 30.1.57.

Hy. 72 Undetermined

A single female was taken in a roadside sample from Matawai on 13.2.57.

Hy. 79 Undetermined

A single female was taken in a paddock sample from Hihitahi on 18.3.57.

Fam. ENCYRTIDAE

The Encyrtidae are parasites of the ova, larvae, and pupae of many orders of insects but more especially the Hemiptera-Homoptera and Lepidoptera. The family is of great economic importance in the field of biological control.

Hy. 51 ? *Ectroma* sp.*

Single specimens (6 females, 3 males) were taken in paddock samples from Victoria Valley (Kaitaia) 16.1.57, Dargaville 22.1.57, Pukekohe 23.1.57, Te Kauwhata 23.1.57, Gisborne-Tiniroto 12.2.57, Mohaka River (Napier-Taupo) 12.2.57, Opotiki-Whakatane 14.2.57, and Te Matai 4.3.57, and in a roadside sample from Mangapakeha on 25.3.57. G. J. Kerrich (pers. comm.) indicates that the host is probably a mealy bug.

Hy. 38 ? *Ericydnus* sp.*

This species (23 females, 4 males) was taken in 12 paddock (14 specimens) and 10 roadside (13) samples. It is widely distributed in the North Island (Fig. 11). Host relationships are not known. It was taken in January (2 samples), February (12), March (5), and early April (3).

Hy. 27 *Rhopus* sp.*

This species (9 females, 3 males) was taken in paddock samples from Victoria Valley (Kaitaia) 16.1.57 (1 specimen), Ruakaka 21.1.57 (2), Gisborne-Tiniroto 12.2.57 (1), Waikohu 13.2.57 (2), and Te Pohue 15.2.57 (1), and in roadside samples from Helensville 23.1.57 (1), Paekakariki 31.1.57 (2), Te Matai 4.3.57 (1), and Hunterville 27.3.57 (1). G. J. Kerrich (pers. comm.) has indicated that the genus is parasitic on mealy bugs.

Hy. 67. Undetermined

A single female was taken in a paddock sample from Otaki on 31.1.57.

Hy. 80 Undetermined

Single females were taken in paddock samples from Ohakune 18.3.57, and Palmerston North (Pahiatua Track) 25.3.57.

Fam. EULOPHIDAE

This family affects almost all orders of insects either as primary or secondary parasites. Many species are highly beneficial biological control agents, e.g. the North American species *Aphelinus mali* Hald. has been introduced into New Zealand and is now largely responsible for the effective reduction in numbers of the woolly aphid which was once a very serious pest in apple orchards.



Fig. 11



Fig. 12

Fig. 11.—Distribution map for the encyrtid ? *Ericydnus* sp. Hy. 38.

Fig. 12.—Distribution map for the eulophid *Aprostocetus* sp. Hy. 35.

Hy. 53 *Aphelinus* sp.

This species (6 females, 1 male) was taken in paddock samples from Gisborne-Tiniroto 12.2.57 (1 specimen) and Taihape 18.3.57 (1), and in roadside samples from the last named (1) and Waverley 24.1.57 (4). Host relationships are not known.

Hy. 27A *Aphytis* sp.*

Two females were taken in a paddock sample from Ruakaka on 21.1.57. It is probably parasitic on a scale insect.

Hy. 35 *Aprostocetus* sp.*

This species (401 females, 4 males) was taken in 27 paddock (107 specimens) and 36 roadside (298) samples, the greatest catch (52) being in a roadside sample from Kai Iwi taken on 24.1.57. This species is widely distributed in the North Island (Fig. 12). G. J. Kerrich (pers. comm.) indicates that this species is probably parasitic on a cecidomyid. It was taken in January (33 samples), February (14), March (15), and early April (1).

Hy. 69 *Chrysocharis* sp.

Gourlay (1930) records *Chrysocharis* sp. as a parasite of *Phytomyza atricornis* Meig. The present species (all females) was taken in paddock samples from Punakitere 19.1.57 (1 specimen), Horotiu 23.1.57 (2), Pukekohe 23.1.57 (1), Matawai 13.2.57 (1), Opotiki 14.2.57 (1),

Okere 4.3.57 (1), and Whitianga 6.3.57 (1), and in roadside samples from Horotiu 23.1.57 (1), Paekakariki 31.1.57 (1), and Norsewood 11.2.57 (2). G. J. Kerrich (pers. comm.) indicates that this species will be parasitic on Lepidoptera or leaf-mining Diptera.

Hy. 21 *Closterocerus* sp.*

This species (all females) was taken in 7 paddock (10 specimens) and 17 roadside (26) samples, the greatest catch (5) being from a roadside sample taken at Marton on 27.3.57. It is widely distributed in the North Island (Fig. 13). G. J. Kerrich (pers. comm.) indicates that this species will be parasitic on Lepidoptera or leaf-mining Diptera. It was taken in January (13 samples), February (5), and March (6).

Hy. 25 Gen. near *Di cladocerus**

This species (all females) was taken in paddock samples from Warkworth 22.1.57 (1 specimen) and Taihape 18.3.57 (7), and in roadside samples from Helensville 23.1.57 (1), Opotiki-Whakatane 14.2.57 (4) and Hikateruru 6.3.57 (1). G. J. Kerrich (pers. comm.) indicates that this will be parasitic on Lepidoptera or leaf-mining Diptera.

Hy. 35A *Euderus* sp.*

A single female was taken in a roadside sample from Palmerston



Fig. 13



Fig. 14

Fig. 13.—Distribution map for the eulophid *Closterocerus* sp. Hy. 21.

Fig. 14.—Distribution map for the eulophid *Hemiptarsenus* sp. Hy. 3.

North (Pahiatua Track) on 25.3.57. G. J. Kerrich (pers. comm.) indicates that this is probably parasitic on a species of *Coleophora* (Lepidoptera).

Hy. 3 *Hemiptarsenus* sp.*

This species (252 females, 306 males) was taken in 69 paddock (455 specimens) and 36 roadside (103) samples, the greatest catch (33) being in a paddock sample from Himatangi taken on 30.1.57. This species is widely distributed in the North Island (Fig. 14). G. J. Kerrich (pers. comm.) indicates that this species is probably parasitic on leaf-mining Diptera or Lepidoptera. It was taken in January (35 samples), February (25), March (41), and early April (4).

Hy. 15 Undetermined

A single specimen was taken in a paddock sample from Himatangi on 30.1.57.

Hy. 21A Undetermined

Two specimens were taken in a roadside sample from Otaki on 31.1.57.

Fam. TRICHOGRAMMATIDAE

Insects belonging to this family are egg parasites mainly of Lepidoptera, Hemiptera, and Odonata, all being very small species. In New Zealand, *Trichogramma* has been recorded from codling moth and *Austrotortrix postvittana* (Walk.) (Tillyard 1926, Gourlay 1930, Dumbleton 1932).

Hy. 32 *Trichogramma* sp.

This species (all females) was taken in paddock samples from Tutira 11.2.57 (2 specimens), Te Pohue 15.2.57 (3), and Paeroa 5.3.57 (1), and in roadside samples from Tutira 11.2.57 (1), Gisborne-Tini-rotu 12.2.57 (1), Rangipo 4.3.57 (3), Mangaweka 18.3.57 (1), and Marton 27.3.57 (1).

Hy. 70 Undetermined

A single female was taken in a roadside sample from Te Pohue on 15.2.57.

Hy. 83 Undetermined

Two females were taken in a paddock sample from Tutira on 11.2.57.

Fam. ELASMIDAE

Insects in this family parasitise Diptera, Hemiptera, and Lepidoptera.

Hy. 34 *Elasmus* sp.

The genus is of world-wide distribution. The present species (16 females, 31 males) was taken in 7 paddock (11 specimens) and 25 roadside (36) samples. It is of widespread occurrence in the North Island (Fig. 15). Host relationships are not known. It was taken in January (9 samples), February (6), March (16), and early April (1).

Fam. MYMARIDAE (Fairy Flies)

All species in this family are egg parasites, and they include some of the smallest known insects. The Hemiptera appear to be the main hosts, although Coleoptera and Lepidoptera are also parasitised.

Hy. 82 *Gonatocerus* sp.*

The genus is well represented in Australia. In the present survey it was taken (all females) in paddock samples from Taupo 4.3.57 (1 specimen), Morrinsville 5.3.57 (2), and Hunterville 18.3.57 (1), and in roadside samples from Te Kuiti 24.1.57 (1) and Waikohu 13.2.57 (1). G. J. Kerrich (pers. comm.) states that "This is probably parasitic on a leaf-hopper egg."

Hy. 75 Undetermined

This species (9 females, 1 male) was taken in a paddock sample from Kohinui 28.3.57 (1 specimen), and in roadside samples from the same site (3) and from Te Pohue 15.2.57 (6).

Hy. 81 Undetermined

A single specimen was taken in a paddock sample from Kohinui on 28.3.57.



Fig. 15



Fig. 16

Fig. 15.—Distribution map for the elasmid *Elasmus* sp. Hy. 34.

Fig. 16.—Distribution map for the scelionid *Baeus seminulum*. Hy. 1.

PROCTOTRUPOIDEA**Fam. SCELIONIDAE**

All scelionids are egg parasites, the hosts being mainly Hemiptera, Lepidoptera, and Orthoptera. Although the relative paucity of the New Zealand fauna is likely to be marked, it may not be so great as indicated by Tillyard (1926) when he recorded only 2 species for this country. In the present account there is reference to some 20 species.

Hy. 1 *Baeus seminulum* Hal.****Distribution***

G. E. J. Nixon (pers. comm.) states "this is almost certainly introduced." This species (apparently all females) was taken in 18 paddock (38 specimens) and 16 roadside (43) samples, the greatest catch (9) being taken in a roadside sample from Taueru on 25.3.57. It is of widespread occurrence in the North Island (Fig. 16).

Habits and Economic Significance

Host relationships are not known. It was taken in January (6 samples), February (6), March (21), and early April (1).

Hy. 1A ? *Baeus* sp.*

A single female was taken in a roadside sample from Maramarua on 6.3.57.

Hy. 16 *Ceratoteleia* sp.*

Single specimens of this species (2 females, 3 males) were taken in paddock samples from Puketona 18.1.57, Uruti 24.1.57, Maramarua 6.3.57, and Whitianga 6.3.57, and in a roadside sample from Pukekohe 12.3.57. Host relationships are not known.

Hy. 84 Gen. near *Ceratoteleia**

This species (all males) was taken in paddock samples from Victoria Valley (Kaitia) 16.1.57 (1 specimen), Punakitere 19.1.57 (1), and Tiniroto 12.2.57 (2), and in roadside samples from Maramarua 8.3.57 (1) and Te Kuiti 8.3.57 (1). Host relationships are not known.

Hy. 33 *Macroteleia* sp.*

The genus is recorded from Australia (Tillyard 1926). In the present survey the species (1 female, 3 males) was taken in paddock samples from Dargaville 22.1.57 (1 specimen), and Opotiki-Whakatane 14.2.57 (1), and in a roadside sample from Waitoa on 5.3.57 (2). Harford (1958) figures a parasite from the eggs of the cicada *Melampsalta cingulata* (Fabr.) which may well be a species of *Macroteleia*.

Hy. 2 *Microphanurus* sp.

The genus is of world-wide distribution. This species (15 females, 12 males) was taken in 7 paddock (15 specimens) and 9 roadside (12) samples. It is of widespread occurrence in the North Island (Fig. 17).

Microphanurus basalis Woll. was introduced into New Zealand in 1949 (Cumber 1951) to assist control the green vegetable bug *Nezara viridula* (L.) and is now widely distributed in the North Island and present in the north of the South Island. The genus, which is commonly parasitic on the eggs of pentatomids, was known to be present in New Zealand before the introduction of *Microphanurus basalis*. In 1949 a species from *Cuspicona simplex* Walk. which could not be induced to parasitise the eggs of *Nezara viridula* was found and bred at Paihia, Bay of Islands. Eggs of *Rhopalimorpha* are also known to be parasitised by a species of *Microphanurus* (Pendergrast 1952).

There are several potential pests amongst our indigenous pentatomids, and these are doubtless controlled in some measure by this genus. The most common pasture pentatomid is *Dictyotus caenosus* (Westw.) and this is the likely host of the present species. It was taken in January (4 samples), February (10), and March (2).

Hy. 86 ? *Microphanurus* sp.

A male was taken in a paddock sample from Gisborne-Tiniroto 12.2.57, and a female in a roadside sample from Kai Iwi on 24.1.57.

Hy. 6 *Synopeas* sp.*

This species (11 females, 8 males) was taken in 9 paddock (13 specimens) and 4 roadside (6) samples. It is widely distributed in the North Island (Fig. 18). Host relationships are not known. It was taken in January (2 samples), February (5), and March (6).



Fig. 17



Fig. 18

Fig. 17.—Distribution map for the scelionid *Microphanurus* sp. Hy. 2.

Fig. 18.—Distribution map for the scelionid *Synopeas* sp. Hy. 6.

Hy. 42 *Teleas* sp.*

This species (all males) was taken in a paddock sample from Foxton 4.4.57 (1 specimen), and in roadside samples from Alton 24.1.57 (1) and Tolaga Bay 12.2.57 (2). Host relationships are not known.

Hy. 18 *Telenomus* sp.*

The genus is recorded from Australia (Tillyard 1926). This species (5 females, 9 males) was taken in paddock samples from Tutira 11.2.57 (1 specimen), Gisborne-Tiniroto 12.2.57 (3), Matawai 13.2.57 (2), Whakatane 14.2.57 (1), and Te Matai 4.3.57 (2), and in roadside samples from Gisborne-Tiniroto 12.2.57 (2), Gisborne 13.2.57 (2), and Taupo-Rotorua 4.3.57 (1). Host relationships are not known. The eastern distribution in the North Island is of interest.

Hy. 18A *Telenomus* sp.*

This species (12 females, 21 males) was taken in 12 paddock (23 specimens) and 7 roadside (10) samples. It is of widespread occurrence in the North Island (Fig. 19). Host relationships are not known. It was taken in February (9 samples) and March (10).

Hy. 8 *Trimorus* sp.*

This species (1 female, 19 males) was taken in paddock samples from Tutira 11.2.57 (4 specimens), Matawai 13.2.57 (14), and Te Pohue 15.2.57 (1), and in a roadside sample from Matawai 13.2.57 (1). Host relationships are not known.

Hy. 42A *Trimorus* sp.*

This species (1 female, 7 males) was taken in paddock samples from Te Awamutu 23.1.57 (1 specimen), Gisborne-Tiniroto 12.2.57 (1), and Kohinui 28.3.57 (1), and in roadside samples from Gisborne-Tiniroto 12.2.57 (1), Gisborne 13.2.57 (3), and Maramarua 6.3.57 (1). Host relationships are not known.

Hy. 31 Undetermined (Baeinae)

This species (3 females, 1 male) was taken in a paddock sample from Whangaroa 17.1.57 (1 specimen), and in roadside samples from Tutira 11.2.57 (1), Wairoa 12.2.57 (1), and Waitoa 5.3.57 (1).

Hy. 46 Undetermined

Single females were taken in roadside samples from Mangapakeha 25.3.57, Masterton 25.3.57, and Tarata 2.4.57.

Hy. 48 Undetermined

A male was taken in a paddock sample from Ohaewai on 19.1.57, and a female in a roadside sample from Otaki on 31.1.57.

Hy. 54 Undetermined

Single males were taken in paddock samples from Wellsford 22.1.57, and Norsewood 11.2.57,

Hy. 60 Undetermined

A female was taken in a paddock sample from Eketahuna on 25.3.57, and a male in a paddock sample from Hihitahi on 18.3.57.

Hy. 85 Undetermined

A male was taken in a paddock sample from Whangaroa on 17.1.57.

Hy. 87 Undetermined

Single females were taken on 6.3.57 in paddock samples from Whitianga and Hikateruru, and in roadside samples from Coromandel and Hikateruru.



Fig. 19



Fig. 20

Fig. 19.—Distribution map for the scelionid *Telenomus* sp. Hy. 18A.

Fig. 20.—Distribution map for the diapriid *Spilomicrus* sp. Hy. 19.

Fam. PLATYGASTERIDAE

Most species belonging to this family lay their eggs in those of the Cecidomyiidae, but the parasite does not develop until after the eclosion of the larval cecidomyiid.

Hy. 6A *Platygaster* sp.

Platygaster minuta Zett. is recorded in New Zealand as a parasite of *Mayetiola destructor* Say. (Gourlay 1930). The present species (2 females, 13 males) was taken in paddock samples from Eltham 24.1.57 (1 specimen), Piopio 24.1.57 (1), Waitoa 5.3.57 (1), Kaimai 5.3.57 (2), Ohakune 18.3.57 (1), and Tarata 2.4.57 (2), and in roadside

samples from Gisborne 13.2.57 (2), Waikohu 13.2.57 (1), Taupo-Rotorua 4.3.57 (3), and Manaia (Taranaki) 3.4.57 (1). Host relationships are not known.

Hy. 73 Undetermined

Single females were taken in paddock samples from Wairoa on 12.2.57 and Te Pohue on 15.2.57.

Fam. DIAPRIIDAE

The Diapriidae are mainly parasites of Diptera.

Hy. 13 *Loxotropa* sp.*

This species (2 females, 7 males) was taken in paddock samples from Gisborne 13.2.57 (1 specimen), Taueru 25.3.57 (1), and Porangahau 26.3.57 (4), and in roadside samples from Taueru 25.3.57 (2), and Hunterville 27.3.57 (1). Host relationships are not known.

Hy. 19 *Spilomicrus* sp.

The genus is recorded by Tillyard (1926) as being present in New Zealand. The species (11 females, 32 males) was taken in 19 paddock (27 specimens) and 9 roadside (16) samples. It is widely distributed in the North Island (Fig. 20). Host relationships are not known. It was taken in January (7 samples), February (6), and March (15).



Fig. 21

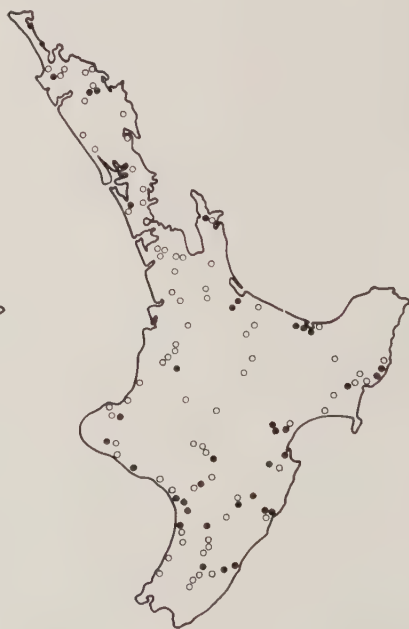


Fig. 22

Fig. 21.—Distribution map for the diapriid *Trichopria* sp. Hy. 12.

Fig. 22.—Distribution map for the ceraphronid *Aphanogmus* sp. Hy. 7.

Hy. 12 *Trichopria* sp.*

The genus occurs in Australia. This species (194 females, 67 males) was taken in 62 paddock (226 specimens) and 24 roadside (35) samples, the greatest catch (20) being from a paddock sample taken at Masterton on 25.3.57. It is widely distributed in the North Island (Fig. 21). Host relationships are not known. It was taken in January (24 samples), February (18), March (40), and early April (4).

Fam. CERAPHRONIDAE

Imms (1957) states "The Ceraphronidae attack insects of several orders but are probably most generally secondary parasites of aphids or coccids through various Braconids and Chalcid primaries." The family appears to have been first recorded in New Zealand by Gourlay (1930) who indicated *Lygocerus niger* How. as parasitic on *Aphidius rapae* Curt.

Hy. 7 *Aphanogmus* sp.*

The genus is present in Australia. This species (35 females, 53 males) was taken in 25 paddock (56 specimens) and 14 roadside (32) samples, the greatest catch (8) being from a paddock sample taken at Tolaga Bay on 12.2.57. It is widely distributed in the North Island (Fig. 22).

In view of the probable secondary parasitism on aphids, the species is to be regarded as an undesirable one. It was taken in January (7 samples), February (18), March (12), and early April (2).

Hy. 74 *Aphanogmus* sp.*

This species (6 females, 2 males) was taken in paddock samples from Himatangi 30.1.57 (1 specimen), Napier 11.2.57 (1), Opotiki 14.2.57 (1), Te Pohue 15.2.57 (2), Okere 4.3.57 (1) and Wallingford 26.3.57 (1), and in a roadside sample from Ohaewai 19.1.57 (1).

Hy. 57 *Lygocerus* sp.

This species (8 females, 27 males) was taken in 17 paddock (32 specimens) and 3 roadside (3) samples, the greatest catch (13) being in a paddock sample from Levin taken on 31.1.57. It is of widespread occurrence in the North Island (Fig. 23). It is probably associated with aphids—possibly *Rhopalosiphum padi* (L.), which is the common grass-frequenting species. It was taken in January (8 samples), February (7), and March (5).

Hy. 43 Undetermined

Single specimens (1 female, 2 males) were taken in a paddock sample from Pukekohe 7.3.57, and roadside samples from Silverdale 23.1.57, and Pakipaki 11.2.57.

BETHYLOIDEA

Fam. BETHYLIDAE

Insects in this family parasitise the larvae of Coleoptera, Diptera, and Lepidoptera. Tillyard (1926) records two species in New Zealand belonging to the genera *Sierola* and *Apanesia*.



Fig. 23



Fig. 24

Fig. 23.—Distribution map for the ceraphronid *Lygocerus* sp. Hy. 57.

Fig. 24.—Distribution map for the formicid *Monomorium antarcticum*. Hy. 45.

Hy. 62 Undetermined

A single female was taken in a paddock sample from Mohaka River (Napier-Taupo) on 15.2.57.

FORMICOIDEA

Fam. FORMICIDAE

A recent review of the ants of New Zealand (Brown 1958) has provided information from which much of the following biological notes are drawn.

Hy. 37A *Amblyopone saundersi* Forel

Distribution

This species which has close relatives in Australia "is very widely distributed in New Zealand, and may include all the more temperate forest country of both main Islands." (Brown 1958). A single male was taken in a paddock sample from Eketahuna on 25.3.57.

Habits and Economic Significance

This is evidently mainly a forest-frequenting species and is not important in pastures,

Hy. 26 *Iridomyrmex glaber* (Mayr)*Distribution*

Brown (1958) states in reference to this cosmopolitan species—"In New Zealand, *I. glaber* does not seem to be very common at present. Two or three small lots have been seen from Auckland and suburbs, and the species may well occur in other cities. No samples have been received from wild areas of the country." In the present survey single workers were taken in a paddock sample from Dargaville 22.1.57, and in roadside samples from Helensville 23.1.57, and Opotiki-Whakatane 14.2.57. A queen was taken in a roadside sample from Ruakaka on 21.1.57.

Habits and Economic Significance

Brown (1958) continues "Since this ant attends homopterans and is at home in domestic environments, it should be regarded as a potential pest."

Hy. 45 *Monomorium antarcticum* (White)*Distribution*

This is probably the most common and widely distributed New Zealand species. It was taken in 17 paddock (42 specimens) and 11 roadside (18) samples, and included queens, males, and workers. It is of widespread occurrence in the North Island (Fig. 24).

Habits and Economic Significance

Nests are found in the soil, under stones, etc., in a "wide variety of ecological situations, ranging from warmer forests in the North to cold bogs at high altitudes in the South, and open pastures of all kinds throughout New Zealand at lower altitudes. The ants are general feeders . . . and tend homopterans." (Brown 1958). Their importance in pastures is difficult to gauge.

Hy. 37 *Ponera eduardi* Forel*Distribution*

Brown (1958) regards this as an introduced species and records it "from the vicinity of ports and other population centres, especially Auckland, with the Coromandel Peninsula, Wellington, and Nelson. There are other records from the North Island to show that the species is successful in expanding its range within the country." In the present survey this species was taken in paddock samples from Alton 24.1.57 (3 specimens), Kai Iwi 24.1.57 (2), and Mapiu 8.3.57 (2), and in roadside samples from Mapiu 8.3.57 (1), and Porangahau 26.3.57 (1).

Habits and Economic Significance

"Apparently the diet consists mainly of soil-inhabiting arthropods such as Collembola." (Brown 1958.)

Hy. 59 *Prolasius advena* (F. Smith)

A male was taken in a roadside sample from Te Kuiti on 8.3.57. "The species is widespread in both Islands where it nests primarily in bush country." (Brown 1958.)

APOIDEA

This super-family includes the solitary and social bees which play such an important part in plant pollination. The habits of the families are well known.

Fam. HALICTIDAE

Hy. 66 *Halictus* sp.

Single specimens (1 female, 2 males) were taken in roadside samples from Eltham 24.1.57, Matawai 14.2.57, and Coromandel 6.3.57.

Fam. APIDAE

Hy. 55 *Apis mellifica* L. (Honey Bee)

This species, the common honey bee, was taken at many of the sampling sites throughout the North Island. In most cases, to facilitate handling, samples which obviously contained such bees were discarded in favour of another sample.

Hy. 65 *Bombus ruderatus* (Fabr.)

Two workers were taken in a roadside sample from Piopio on 24.1.57. This species is of widespread occurrence in the North Island.

DISCUSSION

This account of the Hymenoptera associated with pastures in the North Island emphasises two things. Firstly, it emphasises the complexity of the association of insects which commonly inhabits our pastures. Most of the hymenopterous species are parasitic on other species of insects, and are dependent upon them for their existence. Some species, to heighten the complexity, are themselves parasitic on these parasites. Secondly, this survey emphasises the inadequacy of our knowledge of the New Zealand Hymenoptera. In the present account 31 genera are first records for New Zealand.

The importance of each parasitic species cannot be gauged until the host relationships have been ascertained, and many of these are likely to remain unknown for some time. It should be emphasised that the numerical strength of a species bears no relationship to its potential, for in host/parasite associations the balance is often maintained at low population levels.

There can be no doubt that many of the insects associated with our pastures are potential pests and that they are held in check by these very valuable biological control agents which are invariably present in some measure. A knowledge and recognition of this pasture insect complex will assist in explaining changes in insect populations associated with varying weather conditions and varying agricultural practices.

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THE INSECT COMPLEX OF SOWN PASTURES IN THE NORTH ISLAND

VI. THE PSOCOPTERA AND NEUROPTERA AS REVEALED BY SUMMER SWEEP-SAMPLING

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Summary

A sweep-sampling survey during the summer of 1957 revealed 7 species of Psocoptera and 1 of Neuroptera in the main pasture types occurring in the North Island. The identity, distribution, and abundance of these species are indicated, and their possible economic importance is discussed.

INTRODUCTION

During the summer of 1957, a survey of insects associated with pastures in the North Island was carried out. In an earlier paper (Cumber 1958) techniques, coverage, and relevant sampling station details are given, and the insect complex generally as shown by sweep-sampling is analysed and discussed. The information in respect of the Hemiptera (Cumber 1959a), Diptera (Cumber and Harrison 1959), Coleoptera (Cumber 1959b) and Hymenoptera (Cumber 1959c) has already been indicated.

In the present account the Psocoptera and Neuroptera are discussed with regard to the identity of the species, their distribution, numbers encountered, and possible economic importance.

The survey involves 113 paddock and 108 adjacent roadside samples each comprising a single sweep from an area of approximately 8 square yards.

Distribution maps for 2 species are presented, the presence of a species being indicated by a black dot in contrast to a ring where sampling failed to reveal its presence.

RESULTS

Order **PSOCOPTERA**

Fam. CAECILIIDAE

P. 1 *Caecilius flavistigma* Tilly.

Distribution

This New Zealand species was described by Tillyard in 1923 from "Nelson, common throughout the summer months, both in native bush and in orchards; Hamilton." In the present survey it was taken in 10 paddock (12 specimens) and 8 roadside (9) samples. It is of widespread occurrence in the North Island (Fig. 1).

Habits and Economic Significance

It is not of importance in pastures. It was taken in January (7 samples), February (2), March (8) and early April (1).

P. 2 *Caecilius zelandicus* Tilly.

Distribution

This New Zealand species was described by Tillyard in 1923 as "common in native bush in most parts of New Zealand." In the present survey single specimens were taken in roadside samples from Silverdale 23.1.57, Opotiki-Whakatane 14.2.57, and Tauranga 5.3.57.



Fig. 1.—Distribution map for the caeciliid, *Caecilius flavistigma* P.1.

Habits and Economic Significance

It is not of importance in pastures.

P. 3 *Caecilius* sp.

A single specimen of what is apparently an unrecorded species was taken in a paddock sample from Taupo on 4.3.57.

P. 4 *Ectopsocus congener* Tilly.

Distribution

In describing this New Zealand species Tillyard (1923) states that it is "abundant in many localities throughout New Zealand, both in the bush and amongst orchard trees. Exceedingly abundant around Nelson." In the present survey it was taken in roadside samples only from Alton 24.1.57, Te Kuiti 24.1.57, Norsewood 11.2.57, Takapau 11.2.57, Gisborne-Tiniroto 12.2.57, Tolaga Bay 12.2.57, Rangipo 4.3.57, Taupo 4.3.57, and Te Matai 4.3.57.

Habits and Economic Significance

It is not of importance in pastures.

Fam. MYOPSOCIDAE

P. 6 *Myopsocus novae-zealandiae* Kolbe

Distribution

Tillyard (1923) indicates that this species is present throughout New Zealand and in some parts is very common. In the present survey 2 specimens were taken in a paddock sample from Foxton on 4.4.57.

Habits and Economic Significance

This species is not important in pastures. Large colonies are often found under bark, between cracks in wooden fence posts, logs, etc. The sampling site in the present instance was a paddock to the leeward of a pine plantation, and it is surmised that wind was responsible for scattering the insects over adjacent pastures.

P. 5 *Myopsocus* sp.

A single specimen of an unrecorded species was taken in a paddock sample from Tutira on 11.2.57.

Fam. MESOPSOCIDAE

P. 7 *Philotaropsis* sp.

A single specimen of an unrecorded species was taken in a roadside sample from Helensville on 31.1.57.

Order **NEUROPTERA**

Fam. HEMEROBIIDAE

N. 1 *Micromus tasmaniae* Walk.

Distribution

This species is common to Australia and New Zealand. In the

present survey it was taken in 6 paddock (7 specimens) and 5 roadside (7) samples. It is widely distributed in the North Island (Fig. 2).

Habits and Economic Significance

This species was taken in January only, and this accounts for the seemingly western distribution shown in Fig. 2. It is regarded as a highly beneficial biological control agent of aphids and scales, being actively predaceous especially in the larval stages.



Fig. 2.—Distribution map for the hemerobiid. *Micromus tasmaniae* N.I.

DISCUSSION

The only detailed study of the New Zealand psocids is that by Tillyard (1923) who records 19 species. Three of these are cosmopolitan, one a previously described New Zealand species, and the remainder new species.

Apart from the ability of certain species (mainly wingless) to destroy stored materials, the majority are regarded as relatively harmless insects. They occupy a wide range of habitat and many are regarded as being usually associated with fungal growth no doubt being responsible for spore dissemination under such circumstances.

Because of their habits, they are regarded as being of very minor importance in pastures. The disturbed nature of the habitat is not conducive to their build-up. This is reflected in the relative numbers taken in paddock and roadside sweeps, the latter being much more productive.

Only a fraction of the species present in New Zealand have been described. The writer has collected many as yet unrecorded species including representatives of the primitive scaled lepidopsocids.

The single neuropteran species encountered is undoubtedly a beneficial insect but in no cases has even a moderate population been detected, and it has appeared only in January. It is suspected that the figitid species *Anacharis zelandica* Ashm. may be responsible for keeping its numbers in check.

ACKNOWLEDGEMENTS

Assistance in field work given by the staff of the Entomology Division sub-station at Palmerston North is gratefully acknowledged.

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OBSERVATIONS ON THE EFFECTS OF THE ADMINISTRATION OF THIOCYANATE AND THIOURACIL TO SHEEP

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(Received for publication, 13 May 1959)

Summary

Failure to control the plasma level by renal secretion is the cause of the unpredictable toxicity of thiocyanate to sheep.

High levels of methyl thiouracil given to the ewe do not produce goitres in the lambs as large as intermediate levels do. At these high levels iodide may enhance the goitrogenesis, while at lower levels it partially protects against goitre.

The relation in time between the radioiodine concentration test and the duration and time of goitrogen administration has a great effect on the result.

Previous suggestions that carrier iodide could increase the accumulation of radioiodine in the sheep by overcoming the goitrogenic effect of low levels of dietary thiocyanate are not confirmed.

Regular ingestion of thiouracil by the pregnant ewe interferes with the rapid development of foetal thyroid activity found during the fourth month of pregnancy.

INTRODUCTION

In the course of investigations into goitre in sheep it became desirable to distinguish between thiocyanate-like and thiouracil-like goitrogens, and to achieve this end a thorough knowledge of the effects of these two substances on sheep was desirable.

In the first place, as my main interest was in congenital goitre in lambs from ewes grazing on kale, I wished to know whether one or both of the goitrogens, thiouracil and thiocyanate, could cause a similar condition. This was of particular interest since the evidence, so far collected, was conflicting. On the one hand, the goitrogen of kale caused the accumulation of inorganic iodide in the thyroid of the ewe in the manner expected of thiouracil-like goitrogens (Wright and Sinclair 1958), but, on the other hand, the goitre produced in the lamb could be prevented by iodine prophylaxis. This would be expected from a goitrogen with an action like that of thiocyanate. This raised the question: can iodine prophylaxis prevent congenital goitre caused by thiouracil-like agents? A later section of this report describes work on these problems.

In experiments with thiocyanate, an attempt was made to hold the blood thiocyanate of sheep at levels obtainable on natural fodder. Levels of 9–15 mg CNS⁻ per 100 ml serum had been found in animals grazing on cyanogenetic white clover (Butler *et al.* 1957) but, before these levels could be produced by the administration of thiocyanate, dangerous side effects appeared and deaths followed in spite of with-

drawal of the drug. This led to the experiment on the toxicity of thiocyanate reported below.

It has been suggested elsewhere (Wright and Sinclair 1958) that the effect of goitrogens as measured by the accumulation of radioiodine might depend on the length of the treatment period and the timing of treatment relative to the injection of radioiodine. A section of this paper describes work on this point in the special cases of the goitrogens thiouracil and thiocyanate.

In some preliminary work on the accumulation of radioiodine by normal sheep (Wright 1958) it was found that carrier iodide added to the radioiodine increased the accumulation by the thyroid gland instead of decreasing it as was expected. At that time I suggested, as a possible explanation, that the carrier iodide might be overcoming the effect of the thiocyanate found in the blood of sheep on normal feed. This raises the question of whether this "normal" level is in fact goitrogenic.

Further work on this question is reported below.

Finally, an experiment is described in which the effect of thiouracil on the relative concentration of radioiodine by the foetal thyroid was investigated. The foetal sheep develops, during the fourth month, the power to accumulate radioiodine to a level four to five times that of its dam (Wright and Sinclair 1959). Prior to this stage, thyroids of dam and foetus concentrate radioiodine to an equal extent. I thought that a reduction of this high ratio, by goitrogen, might be the means by which the lamb was born goitrous from a clinically normal ewe, and this experiment was made to test this hypothesis.

EXPERIMENTAL METHODS

Measurement of Thyroidal Concentration of Radioiodine in Adult Sheep

The measurements of radioiodine in the experiment with thiouracil and the first thiocyanate experiment were made in the manner already described (Wright 1958). For the other experiments, the Geiger-Mueller tube was replaced by an Ecko 559A scintillation counter using

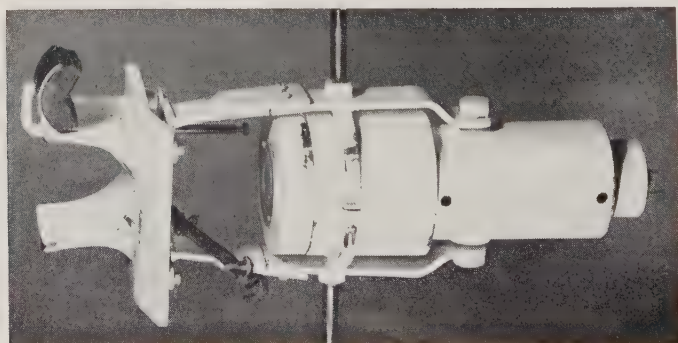


Fig. 1.—The scintillation counter and neck holder.

a $1\frac{1}{2}$ in. crystal. The iron shield was replaced by a lead shield, and collimator and fabricated neck holder used with the G.M. counter was replaced by a cast aluminium version. The new assembly, which fits in the crush already described, is shown in Fig. 1. These modifications enabled the dose to be lowered from 70 to 15 μ c.

Measurement in Foetal Sheep and their Dams

The method described elsewhere (Wright and Sinclair 1959) was used to measure the relative accumulation of radioiodine by the foetal and maternal thyroid glands.

The Administration of Drugs

Tablets of 0.2 g thiouracil and methyl thiouracil were crushed, suspended in water, and given to the sheep by means of a drenching gun.

Thiocyanate solution, which is extremely corrosive and will lead to severe abscess formation if even a very small quantity is allowed to enter the lungs, was always given by stomach tube.

Iodine and iodate solutions were given directly from bottles.

The Estimation of Thiocyanate

Thiocyanate was determined colorimetrically as ferric thiocyanate according to the method of Fister (1950). This author gives a standard curve which does not pass through the 100% transmission point but, if small intervals are used at the low thiocyanate end of the curve, a point of inflection is found and 100% transmission is reached. These two versions are shown in Fig. 2. Both Fister's curve and my curve were obtained on Coleman spectrophotometers and, though Fister states that similar results were obtained with other instruments, the use of a Beckman DU spectrophotometer in my laboratory produced a linear

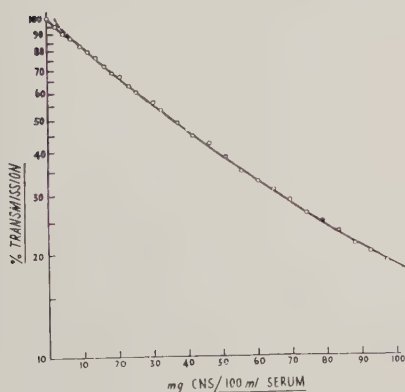


Fig. 2

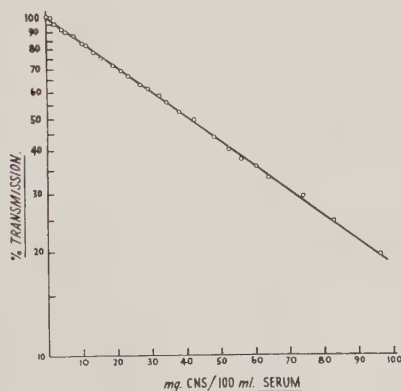


Fig. 3

Fig. 2.—The standard curve for thiocyanate using the Coleman Model II Spectrophotometer. The dotted curve follows Fister's.

Fig. 3.—The standard curve for thiocyanate using the Beckman Model DU.

graph as shown in Fig. 3. In spite of this defect the Coleman instrument was used in this work since it is much faster.

One other modification was found necessary. If the coloured solution was left in the light it faded at a variable rate but, if the colour was allowed to develop in a light-tight box and the transmittance measured within 30 min, reproducible results were obtained.

RESULTS AND DISCUSSION

Toxicity of Thiocyanate

In trying to produce and maintain blood levels of thiocyanate similar to those produced by cyanogenetic fodder, I found that, in some animals, the level would rise suddenly to a high figure and death would follow in a day or so while in others no such effect was seen. I suspected that, provided such crises could be avoided, the sheep would develop a tolerance for quite large amounts of thiocyanate, and decided, therefore, to study the effect of a slowly increasing dose rate in a group of sheep. The experiment is summarised in Fig. 4. In

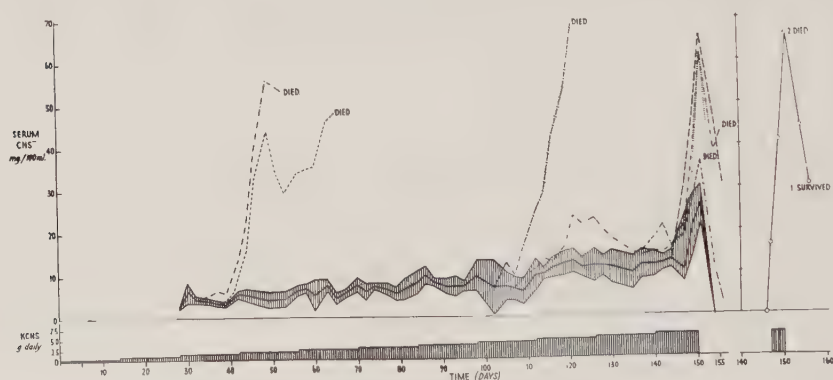


Fig. 4.—The response of serum thiocyanate to increasing dose of KCNS.

this illustration the dosage is shown by the histogram along the bottom while the resulting blood levels are shown by the curve above. The full black line represents the mean value for a group of five sheep which showed no ill effects and whose blood levels returned to normal immediately the drug was withdrawn. The shaded area represents a range of one standard error on either side. Animals whose levels left this shaded area are shown separately. On the right are the mean results from a group of three animals which were put straight on to the maximum dose at the end of the experiment.

Most of the animals tolerated the treatment fairly well but apparently, in some of them, under circumstances which differ from sheep to sheep, the mechanism controlling blood thiocyanate breaks down, the level rises rapidly and usually death follows soon. The three sheep added at the end were unable to adjust themselves and their blood thiocyanates rose rapidly to lethal levels.

During the final days of the experiment urine samples were taken by catheter from four sheep in the main group and all three of the small group, and analysed for thiocyanate. The results are listed in Table 1. The animals which survived in the main group had developed high, and surprisingly similar, levels of excretion, while the fourth sheep which had not developed this ability to excrete thiocyanate to the same extent died later. Of the three introduced on the 147th day, two died almost immediately but the third was still alive when disposed of 2 weeks later.

It is apparent that thiocyanate is controlled by urinary excretion and that quite high rates of intake can be tolerated by many animals provided they are given time to adjust themselves to the increasing dose. These results were very helpful in the design of the later experiments.

TABLE 1. URINARY THIOCYANATES IN MG CNS⁻ PER 100 ML

	147th day	149th day	151st day
<i>Main Group</i>			
Sheep 1	76	169	384
2	60	169	348
3	50	173	364
4 ¹	91	145	216
<i>Subsidiary Group</i>			
Sheep 1 ²	1.6	52	37
2	1.8	69	122
3 ²	1.1	75	Dead

¹Died 163rd day.

²Died 151st day.

Congenital Goitre Induced by Thiocyanate and Thiouracil

Experiments on the induction of congenital goitre by goitrogens were made in both 1957 and 1958. In the first experiment there were four treatment groups of five sheep each. One group received thiouracil with iodate; one without iodate; and one received thiocyanate, the fourth being a control group. Treatment was started on the 94th to 88th day of pregnancy and continued until parturition. In the case of thiocyanate the dose rate was started at 1 g daily and increased by half a gramme every fourth day to 3 g, but as at least three (more probably four) out of five had aborted by the 114th day the dose rate was reduced at this stage to 1 g daily. The iodate treatment was included to show whether or not it could protect the lamb against thiouracil-like goitrogens. The congenital goitre encountered where thousand-headed kale is fed shows some evidence of being caused by such a goitrogen, but is prevented by iodine prophylaxis. Therefore,

it was important to find out whether this was compatible with a thiouracil-like goitrogen. Further details of treatments and the weights of lamb thyroids produced are set out in Table 2.

TABLE 2. CONGENITAL GOITRE FROM THIOCYANATE AND THIOURACIL 1957

Group	Treatment	Weight of Thyroids of Lambs at Birth ¹						Weighted ⁹ Mean g
		Ewe 1 g	Ewe 2 g	Ewe 3 g	Ewe 4 g	Ewe 5 g	Mean ⁸ g	
1.	Control	1.2	1.2	0.8 ² 0.8	1.6 1.1	1.1 1.5	1.3	1.2
2.	KCNS daily (See text for dose.)	29.0	2.2 ³	1.6 ⁴	2.6 ⁵	—	—	
3.	3 g TU daily	49.9 ⁵ 30.5	22.1 ⁶	25.9	32.1 33.2	41.5 29.7	33.1	31.3
4.	3 g TU daily + 370 mg KIO ₃ weekly	31.6	21.2	72.5	10.9 ⁷	14.9	32.4	33.3
		45.6	25.2			29.7		

TU = Thiouracil.

¹ Two figures denote twins.

² Ewe died at 138 days. Not included in mean.

³ Aborted at 114 days.

⁴ Ewe died at 108 days.

⁵ Ewe died at 142 days. Included in mean.

⁶ Ewe died at 140 days. Included in mean.

⁷ Ewe died at 136 days. Not included in mean.

⁸ Lambs are given equal weight.

⁹ Ewes are given equal weight.

Though thiocyanate proved extremely toxic to pregnant sheep, one ewe carried her lamb to term and it showed a greatly enlarged thyroid. This observation is reinforced by the finding that two other foetuses already at the 114th day had glands about twice the normal size at birth. The doses of iodate given were not effective in preventing goitre in the lambs but the doses of both iodate and thiouracil were high and while the former might itself be goitrogenic the latter might be too large to be inhibited by iodine. Consequently, in 1958 I decided to repeat the experiment with lower doses of thiouracil and with only two doses of iodate, as this was the manner found effective against goitre induced by grazing on kale (Sinclair and Andrews 1958) —one dose at the beginning of the fourth and one at the beginning of the fifth month of pregnancy.

Details of treatments and thyroid weights obtained in 1958 are listed in Table 3.

It seems that the goitrogenic effect of methylthiouracil falls off with increasing dose rate though this does not agree with the 1957 experiment. However, it is probable that methylthiouracil, used in 1958 since thiouracil was no longer available, is more effective than

TABLE 3. CONGENITAL GOITRE FROM THIOURACIL 1958

Group	Treatment	Weight of Thyroids of Lambs at Birth ¹						Weighted ⁹ Mean g
		Ewe 1 g	Ewe 2 g	Ewe 3 g	Ewe 4 g	Ewe 5 g	Mean ⁸ g	
1.	Control	1.3 1.4	2.4	1.3 1.3	3.9 ²	1.9 2.1	1.7	1.8
2.	0.5 g MTU daily from 90 days.	104.4	51.0 26.2	47.2 47.7	25.8	54.2 4.6	45.1	49.2
3.	0.5 g MTU daily from 90 days. + 370 mg KIO ₃ at 90 and 120 days.	43.7 34.1	43.8 38.6	5.1 ³ 13.1	35.2	34.0 36.0	31.1	32.7
4.	1.0 g MTU daily from 90 days.	50.5	— ⁵	36.5	10.9	50.5 ⁴ 101.5 ⁴	50.0	43.5
5.	1.0 g MTU daily from 90 days + 370 mg KIO ₃ at 90 and 120 days.	7.6 9.7	30.5	24.4 17.9	14.3	33.2	19.7	21.6
6.	2.0 g MTU daily from 90 days.	4.9	— ⁵	25.5 ⁶ 41.1 ⁶	3.0	11.5 11.9	16.3	13.4
7.	2.0 g MTU daily from 90 days + 370 mg KIO ₃ at 90 and 120 days.	110.4 ⁷	— ⁵	35.6	23.3	46.8 56.6	54.5	55.3

MTU = Methylthiouracil.

¹ Two figures denote twins.² Assisted birth. Head swollen. Not included in mean.³ Foetus long dead in utero. Not included in mean.⁴ Treated 87 days due to mistaken tugging date.⁵ Ewes empty.⁶ Treated 123 days due to mistaken tugging date.⁷ Treated 81 days due to mistaken tugging date.⁸ Lambs are given equal weight.⁹ Ewes are given equal weight.

the unsubstituted compound and the 3 g of the former would be more likely to correspond to 0.5 or 1.0 g of the latter. If this were so, the decrease in gland size at the high level could be due to some systemic toxic effect, though it is difficult to see why this should be inhibited, in the seventh group, by iodine. If the highest level of thiouracil is

neglected there is some indication that iodate affords partial protection against the goitrogenic effect of thiouracil and this will be followed up next season. I arrived at these dose rates by consideration of the possible amount of goitrogen in a day's intake by a grazing animal but it seems that they are far too great and levels in the interval 0.05–0.5 will have to be tried.

Relation of Time of Treatment with Goitrogens to Radioiodine Concentration

Thiouracil. Six groups of five wethers each received the following treatments:

- Groups 1 and 5 — 2 g thiouracil daily from 74 days before to 21 days after the radioiodine injection.
- Groups 2 and 6 — 2 g thiouracil daily from 74 days to 4 days before the radioiodine injection.
- Group 3 — 6 g thiouracil 1 day before the radioiodine injection.
- Group 4 — Controls.

In addition, groups 5 and 6 received 6 g KCNS in 800 ml of water 20 hours after the radioiodine injection.

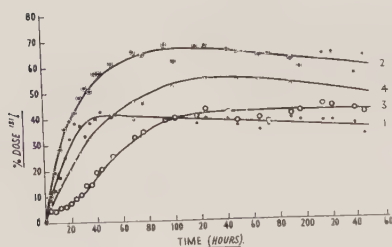


Fig. 5

Fig. 5.—The effect of time of administration of thiouracil on radioiodine absorption in the thyroid gland.

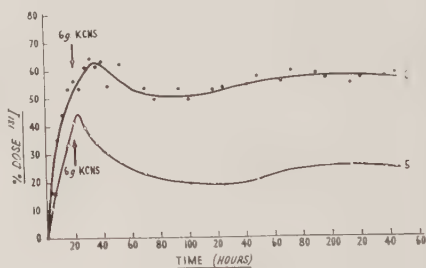


Fig. 6

Fig. 6.—The discharge of iodide from the thyroid gland of thiouracil-treated sheep by means of thiocyanate.

The radioiodine accumulation curves for the first four groups are plotted in Fig. 5. Long exposure to thiouracil (groups 1 and 2) changed the pattern of ^{131}I concentration both in rate and quantity, but though the rate of absorption was the same in both of these groups, that of group 1, which continued to receive the drug during the counting period, reached a maximum much sooner and collected much less total radioiodine than did that of group 2 from which the drug was withdrawn 4 days before. The sheep receiving a single massive dose (group 3) were slow to accumulate radioiodine and, since they showed no signs of releasing thyroxine were probably unable to bind it during the time of measurement.

Fig. 6 shows the results for groups 5 and 6. In the animals of these groups the inorganic iodide of the blocked gland was discharged by thiocyanate. As would be expected, this treatment was more effective in the animals which were still receiving thiouracil than in those from which it had been withdrawn,

Thiocyanate. Two experiments with thiocyanate yielded results very similar to those obtained with thiouracil. The main difference between these two thiocyanate experiments lay in the duration of treatment, the first extending over two months and the second over six. A minor difference was the replacement of the single dose of the first experiment (corresponding to the single dose in the thiouracil experiment) by a treatment spread over six days. The details of these treatments are shown graphically in Fig. 7. Fig. 8 shows the radioiodine curves obtained.

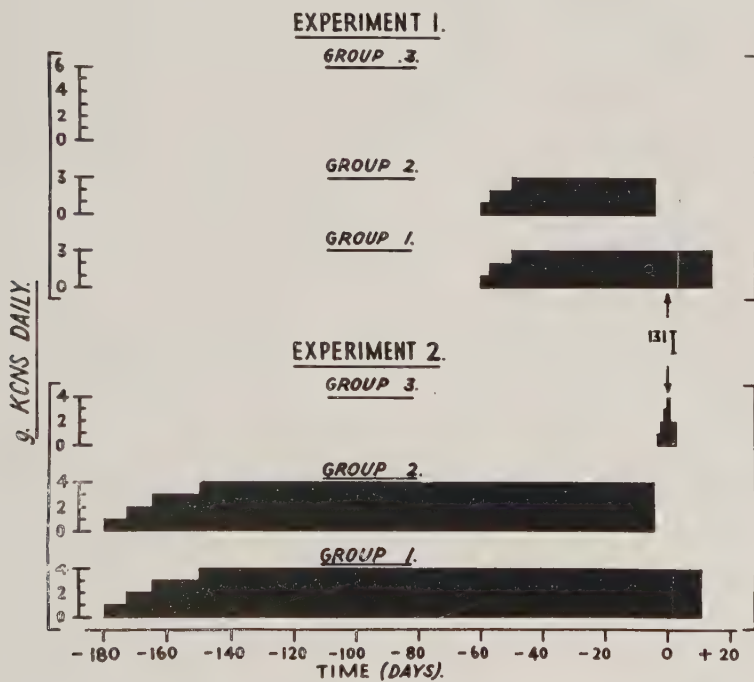


Fig. 7.—The pattern of thiocyanate dosing which produced the results shown in Fig. 8.

Very similar curves resulted from both the long and short experiments when the treatment was uninterrupted (group 1), but when the thiocyanate was withdrawn before the injection of radioiodine (group 2) the rate of discharge of radioactivity was greater in the longer-treated group. There is nothing unexpected in these results but they do emphasise the vast effect that timing and duration of treatment may have on the results.

Effect of Carrier

To test the hypothesis that carrier iodine might inhibit dietary thiocyanate at low levels, four groups of four wethers were given the following treatments:

- Group 1 — 100 g I^- daily.
- Group 2 — 100 g I^- daily + 100 μg carrier I^- added to the ^{131}I .
- Group 3 — 0.5 KCNS daily.
- Group 4 — 0.5 g KCNS daily + 100 μg carrier I^- added to the ^{131}I .

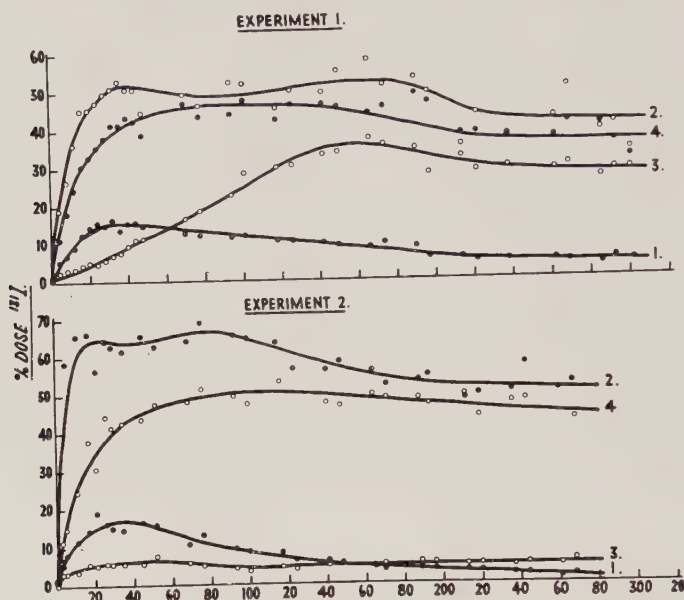


Fig. 8.—The effect of time and period of administration of thiocyanate on radioiodine absorption.

All treatments were given for 82 days before the radioiodine injection and continued during the radioactivity measurements. The KCNS treatment resulted in moderately raised blood thiocyanate levels as shown in Table 4. The results of this experiment shown in Fig. 9 do not support the hypothesis. Though added carrier lowered and thiocyanate raised the level of accumulation of radioiodine, the differences are not statistically significant. This leaves unexplained the increased concentration of radioiodine due to carrier found previously (Wright 1958). The blood levels of the iodide-treated sheep are below the normal level for untreated animals but, as this finding is uncontrolled, it cannot be given any weight. However, the possibility that iodine can affect blood thiocyanate merits further study.

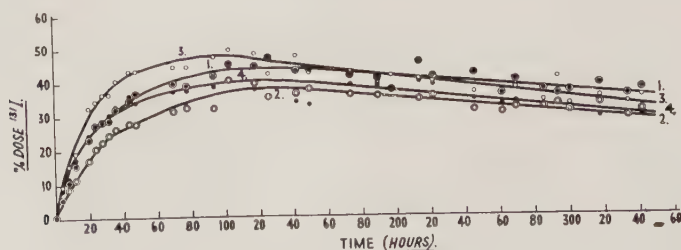


Fig. 9.—The absence of effect of added carrier or of a low level of thiocyanate on radioiodine absorption.

TABLE 4

Days before ¹³¹ I Injection	Mean Serum KCNS in mg CNS ⁻ per 100 ml			
	Group 1	Group 2	Group 3	Group 4
1	0.5	0.7	2.1	2.7
8	1.0	1.0	1.8	2.2
15	1.0	0.3	1.4	1.8
21	0.3	0.2	0.8	0.9
29	0.9	0.7	0.4	1.2
36	0.5	0.6	2.2	1.9
43	0.4	0.3	4.0	3.3
52	0.4	0.2	2.3	2.3
58	0.3	0.2	3.8	2.9
65	0.4	0.3	3.3	3.2
72	0.6	0.3	2.0	1.9

Effect of Thiouracil on Foetal Accumulation of Radioiodine

The pattern of the foetal accumulation of radioiodine relative to the maternal, as described elsewhere (Wright and Sinclair 1959) is marked by a rise in the ratio foetus/ewe from 1 to 4-5 in the fourth month and the occurrence of congenital goitre without clinical goitre in the ewe could be explained by interference with this development of the foetal thyroid. If the foetus and host were effected proportionally one would not expect the great difference seen in the effect of goitrogen intake. For these reasons I ran an experiment in which the ewes were given 2 g thiouracil daily from the 30th day of pregnancy. At various foetal ages the relative radioiodine concentrating power was measured and the figures obtained are set out in Table 5.

TABLE 5. RELATIVE FOETAL ACCUMULATION OF RADIOIODINE

Foetal Age (days)	Foetal ¹³¹ I/g thyroid Maternal ¹³¹ I/g thyroid
61	0.2
89	0.9: 1.6*
99	0.4
104	4.7
110	1.9
116	1.4
130	0.6: 0.4*
138	3.5

* Two figures denote twins.

As I have always found, the drug is variable in its effect from animal to animal, but it is evident that it interferes with this pattern of activity and tends to reduce the ratio to unity.

ACKNOWLEDGEMENTS

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THE INFLUENCE OF TIME OF TILLER ORIGIN AND NITROGEN LEVEL ON THE FLORAL INITIATION AND EAR EMERGENCE OF FOUR PASTURE GRASSES

By J. R. WILSON, Grasslands Division, Department of Scientific and Industrial Research, Palmerston North

(Received for publication, 22 June 1959)

Summary

A study was undertaken for perennial ryegrass, Italian ryegrass, cocksfoot, and timothy, of the floral initiation, ear emergence, leaf number at ear emergence, and percentage of fertile tillers with dates of origin between May 1958 and February 1959. The influence of two levels of nitrogen was examined.

Despite large ranges in their time of origin, winter and early-spring tillers of perennial ryegrass, Italian ryegrass, and timothy all initiated floral primordia within a relatively short period. Ear emergence followed the same pattern.

Mean leaf number at ear emergence decreased with tillers of later origin to minima of 6 for perennial ryegrass, 7 for Italian ryegrass, 9 for cocksfoot, and 14 for timothy.

In cocksfoot and timothy the percentage of fertile tillers was high only for those formed before mid winter, whereas in perennial ryegrass and Italian ryegrass the percentage remained high even for tillers formed up to late spring.

High nitrogen promoted earlier floral initiation and ear emergence, and a greater percentage of fertile tillers. The effect was most marked in cocksfoot.

The majority of heads emerged during the 3-4 week period after the first recorded ear emergence in each species.

The heading of perennial ryegrass was inhibited by a controlled high day/night temperature of 90°F/70°F over a 7-week period.

INTRODUCTION

The grass plant consists of tillers of varying age and stage of development. In the management of grass species for herbage and seed production the relationships between, and time of occurrence of, the vegetative and reproductive phases of these tillers which have originated at different periods of the year are of importance.

Langer (1956) working in the United Kingdom with timothy (*Phleum pratense* L.) found that the ear-bearing capacity of tillers varied with their date of origin. The capacity reached 100% for December-January tillers, this was followed by a steady decline to 1.2% for July tillers, after which time newly formed tillers failed to produce a seed head. Cocks (1958) in the United Kingdom, found abundant heads on timothy plants sown before the end of April, but with later sowings the production of seed heads declined, no heads being formed on plants sown in mid August. Moriya (1956) working in Japan with cocksfoot (*Dactylis glomerata* L.) found that the head-

ing percentage of late-autumn to mid-winter tillers was relatively high, while it was low in early-spring tillers. He concluded that the 1st, 2nd, and 3rd order tillers were the most important in seed production for the first year, and the 4th, 5th, and 6th order tillers for the following year.

Cooper and Saeed (1949) using S.24 perennial ryegrass (*Lolium perenne* L.) found that although there was a difference of six weeks between the time of appearance of the main shoot and of the first axillary tiller, they produced heads within a few days of each other. Langer (1956) found that the time of ear emergence in timothy had a range of only a few days for all tillers arising before the end of April. The time of floral initiation was not examined.

Floral initiation and the morphological development of the growing point have been described for herbage grasses (Sharman 1947). Specific studies have been carried out on ryegrass (Evans and Grover 1940; Cooper 1950, 1951; Jeater 1956), on cocksfoot (Evans and Grover 1940; Gardner and Loomis 1953; Jeater 1956), and on timothy (Evans and Grover 1940). In all these studies the time of floral initiation was established only for the main stem or plant as a whole and not for tillers formed at varying times throughout the year. Determinations of the intervals from floral initiation to ear emergence have been made for the main stem of timothy by Evans and Allard (1934), of cocksfoot by Sprague (1948), and of ryegrasses by Cooper (1952).

Leaf number at ear emergence has received much attention. A minimum vegetative requirement before flowering, measured as leaf number, has been claimed for many grasses and cereals (Purvis and Gregory 1937; Sharman 1947; Cooper and Saeed 1949; Rice 1950; Lamp 1952). This work has largely been on the main shoot although Cooper and Saeed (1949) with Wimmera ryegrass (*L. rigidum* Gaud.) and Lamp (1952) with *Bromus inermis* found that later-formed tillers had lower leaf-number minima at heading. The rate of appearance of new leaves is claimed to be linear (Purvis 1934; Cooper 1951) and the leaf number at ear emergence has been suggested as a means of calculating the date of floral initiation in five range grasses (Rice 1950) and in ryegrasses (Cooper 1951).

The application of nitrogen is a common practice for grass seed production, and its effect on yield has been demonstrated for many species, but its influence on the number and development of fertile tillers has not been as clearly defined.

Evans and Calder (1931) found that nitrogen markedly increased the number of fertile tillers of cocksfoot and less so of perennial ryegrass and timothy. Evans (1934) reported an increase in fertile tillers for timothy and perennial ryegrass and an increase in barren tillers for Italian ryegrass (*Lolium multiflorum* Lam.) and cocksfoot, and later (1937) showed an increase in fertile tillers for cocksfoot and timothy. However Fulkerson *et al.* (1951) reported no such increase for a pasture type of timothy. Evans (1954) showed a slight increase in fertile tillers in cocksfoot, a more marked increase in perennial ryegrass, but a reduction in timothy. Applied nitrogen has been shown to accelerate

flowering in timothy (Evans 1934) and cocksfoot (Gardner 1953), but Purvis (1934) reported little effect on the floral initiation of cereal rye. No effect on the time of ear emergence was observed for ryegrasses (Cooper 1951). Cailahjan (1944) divided plants into three groups according to whether they flower earlier, or later, or are unaffected by nitrogen. His summary does not indicate to which group herbage grasses belong.

The present study on perennial ryegrass, Italian ryegrass, cocksfoot, and timothy was undertaken to provide more information on the development of individual tillers formed throughout the year under both high and low nitrogen conditions. Such information would aid the explanation of results obtained by Sears (1947, 1950), Lambert and Thurston (1952), and Lambert (1956) in field experiments on seed production in New Zealand.

METHODS

The study was carried out at the Palmerston North station of the Grasslands Division of the Department of Scientific and Industrial Research. The species used were perennial ryegrass, Italian ryegrass, timothy, and cocksfoot.

For a representation of each species five plants were chosen at random from New Zealand pedigree strain material. Twenty cuttings, each of 6–10 tillers, from each plant were grown singly in 8 in. pots filled with a 50:50 sand:Manawatu silt loam mixture. These cuttings were planted on 15, 17, 22 May 1958 and 3 June 1958 for Italian ryegrass, perennial ryegrass, cocksfoot, and timothy respectively. With the subsequent large increase in their size it was necessary to transfer them to beds (Manawatu silt loam) on 2 October for perennial and Italian ryegrass, and on 11 November for cocksfoot and timothy. Additional cuttings from the original plants were later divided to replace the former plants in the pots.

Two nitrogen levels in the ratio 6:1 were applied, each to fifty plants per species. The amounts of ammonium nitrate received per plant in equivalents per acre are shown in Table 1.

The aim of the high nitrogen level (6N) was to allow prolific growth and the low nitrogen level (1N) to allow slow growth (Fig. 1).

TABLE 1. NITROGEN APPLICATION PER PLANT (EQUIVALENT 100 lb NH_4NO_3 PER ACRE), FOR EACH SPECIES. (In brackets, growing period in weeks)

Nitrogen Level	Original Plants in Pots		Original Plants in Beds		Replacement Plants in Pots	
	1N	6N	1N	6N	1N	6N
Perennial and Italian ryegrass	1	6 (20)	1	6 (10)	3	18 (28)
Timothy and cocksfoot	2	12 (24)	2	12 (20)	3	18 (22)



Fig. 1.—Comparison between the two nitrogen levels, for each species, on 3 February 1959.

In the pots, nitrogen was applied in weekly dressings, the rates increasing with plant size. The original timothy and cocksfoot plants were in the pots during the period of rapid spring growth and for this reason received a greater quantity of nitrogen (Table 1). In the beds, nitrogen was applied in equal dressings every four weeks. At intervals superphosphate and muriate of potash were applied in adequate quantity for growth.

Every four weeks 100 tillers per nitrogen level per species were tagged, at the two-leaf stage, by means of a loop of coloured plastic wire with aluminium label attached. The two-leaf stage is recognisable very soon after the tiller emerges from the surrounding leaf sheath and has been taken as the time of tiller origin. Tillers were tagged over

the period May 1958-February 1959. After the original plants were transferred to the beds, tagging was continued only on the replacement plants in the pots.

The dates of ear emergence, and the leaf numbers at ear emergence were recorded for each tagged tiller. The dates of floral initiation were determined, for each date of tiller origin, by dissection of ten tillers from each nitrogen treatment, two from each clone. Examinations were carried out for each date of origin at weekly intervals during the spring, and less frequently thereafter. For any one date of tiller origin the first observed date of floral initiation was recorded for each clone and these were used to give a mean date for each species.

After 10 April 1959 all tagged tillers which had not headed were removed and the growing points examined. For perennial ryegrass, Italian ryegrass, and cocksfoot, tillers were classified as vegetative, fertile, or dead. For timothy, two further groups were included: elongated and vegetative, and elongated with head aborted, as classified by Evans (1927).

On 18 December 1958, twenty perennial-ryegrass plants from the high-nitrogen treatment, four from each clone, were placed in controlled-growth cabinets under a 15-hour daylength with a light intensity of 4,000 foot candles (Mitchell 1956). One group of ten plants, two from each of the five clones, was subjected to a day/night temperature of 90°F/70°F and the other to 70°F/50°F. All heads were marked on 24 January 1959 and a count of heads formed after this date was made on 4 March 1959 when the plants were removed from the cabinets.

RESULTS

Daylength and Temperature Conditions

The daylength and temperature conditions during the experimental period are shown in Fig. 2.

The temperature of an unshaded thermocouple 6 in. above the pot surface was measured by a Brown Recorder for the period 22 December 1958 to 7 February 1959 and is shown in Table 2. This was used to give some indication of the temperature at the plant surface.

TABLE 2. MAXIMUM TEMPERATURES 6 in. ABOVE POT SURFACE

Date Period	No. of Days Maximum Temperature was:				
	60-70°F	70-80°F	80-90°F	90-100°F	100-110°F
22 Dec 1958 to 7 Feb 1959	3	14	24	6	1

The pots were watered by sprinkler on most days during this period and thus maximum temperatures as recorded in Table 2 are lower than would be recorded without watering.

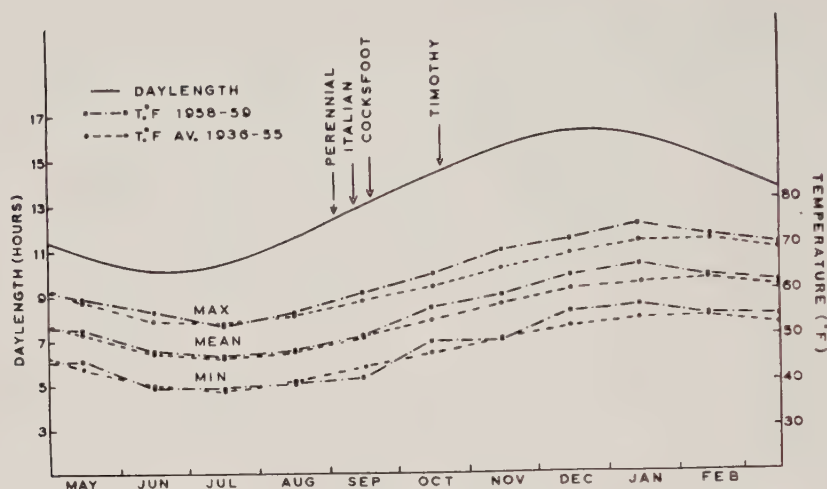


Fig. 2.—The daylength† curve for Wellington* and a comparison between the 1936-55 average and the maximum, mean and minimum monthly temperatures‡ for May 1958-March 1959. The first observed floral initiation in each species is indicated on the daylength curve.

† Including civil twilight.

* Latitude $41^{\circ} 17' \text{ S}$, Palmerston North $40^{\circ} 23' \text{ S}$.

‡ Recorded at Grasslands Meteorological Station, 200 yards distant.

From Fig. 2 it can be seen that the spring and early-summer temperatures are $2-3^{\circ}\text{F}$ above the 1936-55 average.

Floral Initiation

Table 3 shows the date of floral initiation for each date of tiller origin and nitrogen level, for the four species. The figure for any one date of tiller origin is a mean of the first observed date of floral initiation for all clones. The low percentage of flowering tillers and the small number dissected, together with less frequent sampling combine to reduce greatly the accuracy of the date of floral initiation for later-formed tillers. Some clones ceased initiating floral primordia earlier than did others. The number of clones from which each mean date is obtained is shown in brackets in Table 3. Corresponding dates for Figs. 3 and 4 are from the same number of clones. Thus figures for later-formed tillers may be biased in favour of late-flowering clones.

Perennial Ryegrass. Full analysis of the data was possible only up to 5 September after which date some clones failed to initiate. The range in date of tiller origin from 29 May to 12 August (75 days) was reduced to a range of only 9 days in floral initiation.

There was a significant nitrogen effect ($F = 6.85$ for 1, 5 d.f.), the overall means being $1\text{N} = \text{September } 24 \pm 2$, and $6\text{N} = \text{September } 18 \pm 2$, a difference of 6 days.

TABLE 3. FLORAL INITIATION DATE FOR EACH SPECIES, DATE OF TILLER ORIGIN AND NITROGEN LEVEL

Date Tiller Origin	Mean Date Floral Initiation (†)	
	1N	6N
<i>Perennial Ryegrass</i>		
29 May 1958	Sep 16 $\pm$ 2 (5)	Sep 10 $\pm$ 2 (5)
9 Jun "	19 2 (5)	16 2 (5)
1 Jul "	19 2 (5)	17 2 (5)
22 Jul "	22 2 (5)	18 2 (5)
11 Aug "	24 2 (5)	18 2 (5)
5 Sep "	Oct 16 2 (5)	27 2 (5)
6 Oct "	Nov 1 $\pm$ 3 (4)	Nov 1 $\pm$ 3 (5)
3 Nov "	Dec 22 * (2)	27 3 (5)
5 Dec "		Jan 9 * (2)
<i>Italian Ryegrass</i>		
27 May 1958	Oct 4 $\pm$ 3 (4)	Sep 15 $\pm$ 3 (4)
9 Jun "	12 3 (4)	21 3 (4)
30 Jun "	18 3 (4)	29 3 (4)
21 Jul "	18 3 (4)	30 3 (4)
11 Aug "	19 3 (4)	Oct 12 3 (4)
5 Sep "	23 3 (4)	16 3 (4)
6 Oct "	30 $\pm$ 6 (3)	30 $\pm$ 5† (4)
3 Nov "	Dec 1 ‡ 6 (3)	Nov 22 5 (4)
5 Dec "	23 * (2)	Jan 1 5 (4)
<i>Timothy</i>		
10 Jun 1958	Nov 12 $\pm$ 3 (4)	Nov 5 $\pm$ 3 (4)
1 Jul "	14 3 (5)	4 3 (5)
22 Jul "	15 3 (5)	4 3 (5)
12 Aug "	21 3 (5)	13 3 (5)
6 Sep "	28 3 (5)	26 3 (5)
6 Oct "	Dec 22 3 (5)	Dec 9 3 (5)
11 Nov "		29 ‡ (4)
<i>Cocksfoot</i>		
29 May 1958	Sep 30 $\pm$ 6 (5)	Sep 24 $\pm$ 6 (5)
10 Jun "	Oct 6 6 (5)	27 6 (5)
30 Jun "	19 6 (5)	Oct 3 6 (5)
21 Jul "	Nov 2 $\pm$ 8 (4)	5 6 (5)
12 Aug "	20 $\pm$ 7 (3)	16 6 (5)
5 Sep "	Dec 25 7 (3)	Nov 12 6 (5)
6 Oct "	28 * (2)	18 $\pm$ 5 (5)

* No standard errors given as means are of two clones only.

† In brackets, number of clones from which mean is obtained.

‡ Floral Initiation recorded on the same date for all clones, possibly due to insufficient sampling and hence S.E. is either not given or is doubtful.

There was a significant interaction between date of origin and nitrogen level ($F = 5.36$ for 5, 20 d.f.), the effect of high nitrogen on promoting earlier initiation being greatest for the 5 September date of origin.

Italian Ryegrass. Because of poor tillering and an abnormal number of malformed tillers, one clone was removed from the Italian ryegrass experiment.

Data were analysed up to 5 September. The range in date of tiller origin from 9 June to 5 September (88 days) was reduced to a range of only 11 and 26 days in floral initiation for low and high nitrogen levels, respectively.

There was a significant nitrogen effect ($F = 32.72$ for 1, 5 d.f.), the overall means being 1 N — October 16 ± 2 , 6 N — September 30 ± 2 , a difference of 16 days.

There was a significant interaction between date of origin and nitrogen level ($F = 2.93$ for 5, 15 d.f.), the effect of high nitrogen on promotion of earlier initiation being greatest for the last two dates of origin.

Timothy. Data to 6 October were analysed. The range in date of tiller origin from 10 June to 12 August (63 days) was reduced to a range of only 8 days in floral initiation.

There was a significant nitrogen effect ($F = 20.94$ for 1, 4 d.f.), the overall means being 1N — November 24 ± 1 and 6N — November 15 ± 1 , a difference of 9 days. In contrast to the above species the interaction between date of origin and nitrogen level was non-significant.

Cocksfoot. Data up to 5 September were analysed. Initiation date was progressively later with later dates of tiller origin.

There was a significant nitrogen effect ($F = 14.66$ for 1, 5 d.f.), the overall means being 1N — November 2 ± 4 and 6N — October 10 ± 4 , a difference of 23 days.

There was a significant interaction between date of origin and nitrogen level ($F = 3.10$ for 5, 15 d.f.). The effect of high nitrogen on promoting earlier initiation was greater for the later dates of origin.

Ear Emergence

The relationships between date of ear emergence, tiller origin and nitrogen level are shown for each species in Fig. 3.

Perennial Ryegrass. Only a small difference in date of ear emergence was recorded for tillers formed between 29 May and 22 July, later-formed tillers however showed a delay in ear emergence. High nitrogen brought forward the emergence date by approximately 7 days.

Italian Ryegrass. Tillers formed from 27 May to 5 September showed a range in date of ear emergence of only 10 days. Later-formed tillers showed a greater delay in ear emergence. High nitrogen brought forward the emergence date by approximately 7 days.

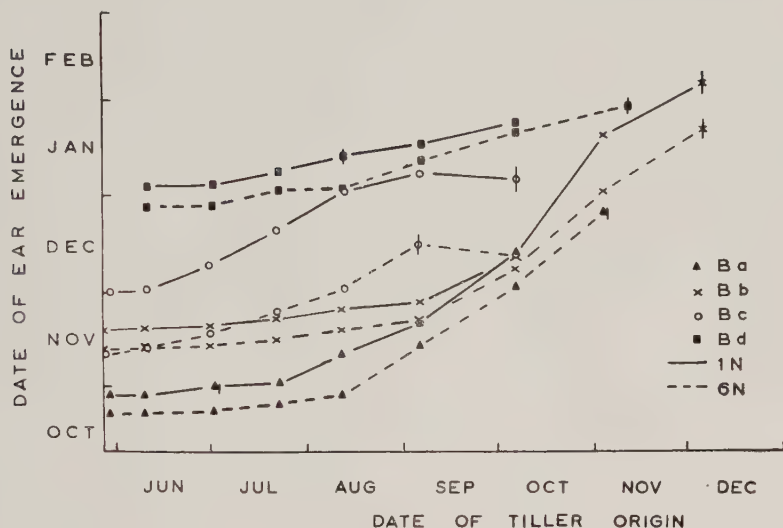


Fig. 3.—The relationship between date of ear emergence, tiller origin and nitrogen level, for each species. Average standard errors are shown for each curve.

Ba = perennial ryegrass, Bb = Italian ryegrass,

Bc = cocksfoot, Bd = timothy

Timothy. Tillers formed from 10 June to 12 August showed a range of only 5 and 9 days in date of ear emergence for the high and low nitrogen levels respectively. Later-formed tillers showed an increasing lateness in date of heading. High nitrogen advanced the date of ear emergence by approximately 6 days.

Cocksfoot. Ear emergence was progressively later with later dates of tiller origin. High nitrogen advanced the emergence date by approximately 20 and 30 days for the early- and late-formed tillers respectively.

Interval to Floral Initiation and Ear Emergence

The number of days from tiller origin to floral initiation and to ear emergence is shown for each nitrogen level and species in Fig. 4.

All Species. The number of days to floral initiation and ear emergence decreased with later dates of tiller origin. The number of days was less under the high nitrogen level. The interval from floral initiation to ear emergence for the early-formed tillers was relatively constant for each species (36-42 days for perennial and Italian ryegrass, 52-60 for timothy), with the exception of cocksfoot under the low nitrogen level where the interval decreased with each successive date of origin. Minima of 30 days for perennial ryegrass, 21 days for Italian ryegrass, 32 days for timothy, and 24 days for cocksfoot were reached in the high-nitrogen treatment. However, the floral initiation recordings for cocksfoot for the last two dates of origin under the low

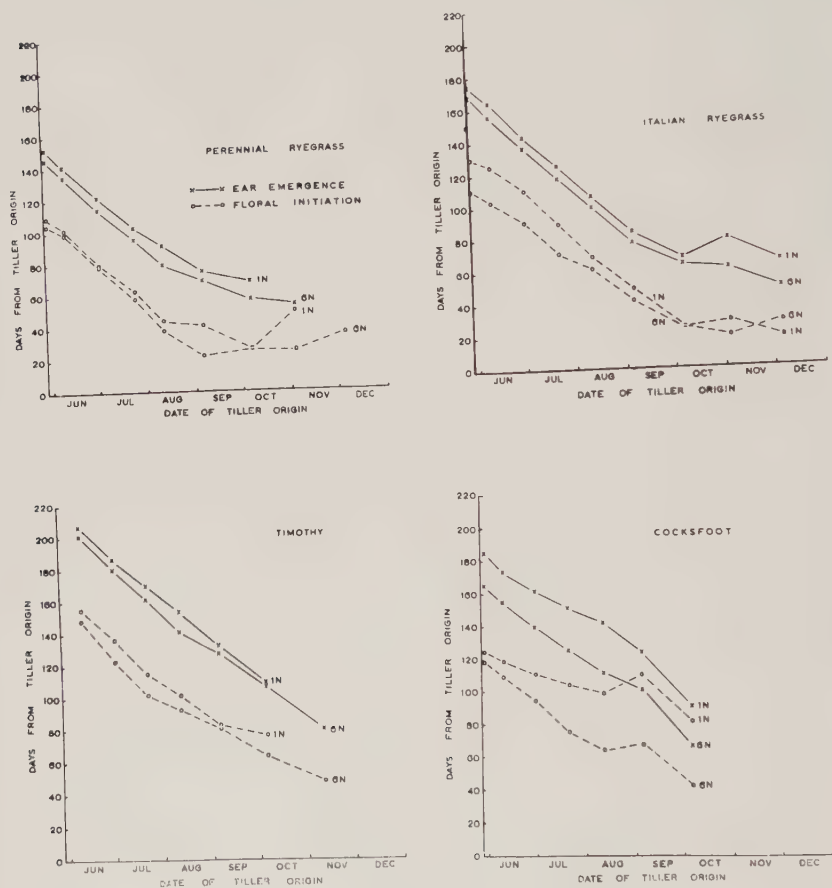


Fig. 4.--Number of days to floral initiation and ear emergence from date of tiller origin for each species at the two nitrogen levels.

nitrogen level are suspect owing to the infrequent sampling, low percentage of floral tillers, and the fact that only two clones are represented in the mean.

Leaf Number at Ear Emergence

The leaf numbers at ear emergence for each species are shown in Table 4 for tillers of varying date of origin under the two nitrogen levels.

Perennial Ryegrass. Leaf number decreased with later dates of tiller origin to a minimum of 6.0 for tillers originating on 6 October, after which a rise was recorded. Tillers formed early showed a higher leaf number under the high nitrogen level.

Other Species. Leaf number decreased with later dates of tiller origin to minima of 7.0 in December for Italian ryegrass, 14 and 9 in

TABLE 4. LEAF NUMBER AT EAR EMERGENCE FOR EACH SPECIES, DATE OF TILLER ORIGIN AND NITROGEN LEVEL

Date of Tiller Origin 1958	Per. Ryegrass		Ital. Ryegrass		Timothy		Cocksfoot	
	1N	6N	1N	6N	1N	6N	1N	6N
27 May	—	—	16.0	15.8	—	—	—	—
29 May	11.4	12.2	—	—	—	—	16.7	16.7
9 Jun	10.7	11.4	14.4	14.1	—	—	—	—
10 Jun	—	—	—	—	21.8	22.4	16.4	16.2
30 Jun	—	—	12.4	12.4	—	—	15.0	14.5
1 Jul	9.2	9.7	—	—	19.1	19.1	—	—
21 Jul	—	—	11.3	11.5	—	—	14.5	13.8
22 Jul	8.2	8.4	—	—	17.5	17.0	—	—
11 Aug	7.3	7.2	11.3	10.7	—	—	—	—
12 Aug	—	—	—	—	16.0	14.3	14.0	12.3
5 Sep	7.3	6.9	10.7	9.8	—	—	13.7	13.1
6 Sep	—	—	—	—	14.9	14.8	—	—
6 Oct	6.3	6.0	8.0	8.3	14.1	14.2	11.5	9.2
3 Nov	—	6.0	8.0	7.3	—	—	—	—
5 Dec	—	8.3	8.0	7.0	—	—	—	—
Av. S.E.	± 0.2	± 0.1	± 0.7	± 0.7	± 0.3	± 0.5	± 0.5	± 0.4

October for timothy and cocksfoot respectively. There were no consistent differences in leaf number between the two nitrogen levels.

Percentage of Fertile Tillers

The percentages of fertile, vegetative and dead tillers are shown in Fig. 5 for each species, date of origin, and nitrogen level.

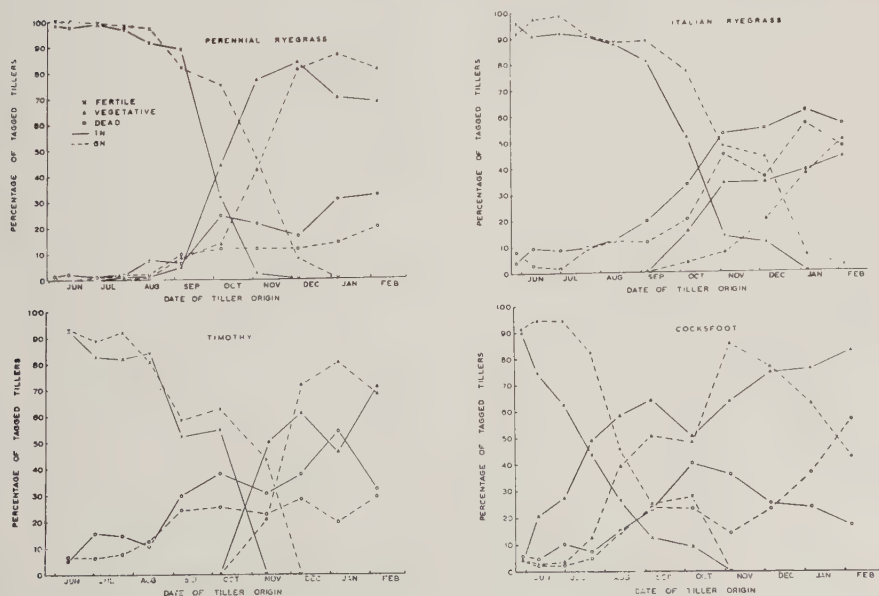


Fig. 5.—Percentages of fertile, vegetative, and dead tillers, for each date of tiller origin.

Perennial Ryegrass. The percentage of fertile tillers remained high for tillers originating between 29 May and 5 September. For later-formed tillers a rapid decline in the percentage of fertile tillers is accompanied by a rapid increase in the percentage of vegetative and a small rise in the percentage of dead tillers. A higher percentage of fertile tillers was recorded under the high nitrogen level for the October, November, and December dates of origin.

Italian Ryegrass. The percentage of fertile tillers remained high for those originating between 27 May and 5 September, after which a decline is evident. The percentage of dead tillers increased for tillers arising during the summer months, reaching a peak of 60% for tillers originating in January. For the later dates of origin a greater percentage of fertile tillers was noted under the high nitrogen level.

Timothy. The percentage of fertile tillers began declining earlier than in the ryegrasses. There was a marked nitrogen effect on percentage of fertile tillers for those arising in November. The data for timothy did not include those tillers which were elongated and vegetative, or those in which the heads aborted after elongation. The percentages for these groups are shown in Table 5.

TABLE 5. PERCENTAGE OF ABNORMAL ELONGATED TILLERS IN TIMOTHY

Date of Tiller Origin	Aborted		Vegetative	
	1N	6N	1N	6N
1958				
10 Jun	2.5			
1 Jul		4.9	1.3	
22 Jul			3.2	
12 Aug	2.7	5.5	2.6	
6 Sep	1.5	7.1	11.9	10.0
6 Oct		6.0	7.1	5.9
11 Nov		3.8	19.6	9.4
8 Dec			1.1	
6 Jan 1959				
5 Feb				

Cocksfoot. The percentage of fertile tillers showed an earlier decline, beginning in May tillers for the low nitrogen treatment and in June for the high nitrogen treatment, this nitrogen effect remaining until October.

Percentage of Heads Emerging per Week

The percentage of tagged heads emerging per week is shown for each species and nitrogen level in Fig. 6.

Perennial Ryegrass. Under the high nitrogen level 77% of the heads emerged during the three-week period after 13 October. Under the low nitrogen level emergence was delayed one week. The majority of the tillers at both nitrogen levels had emerged by mid November.

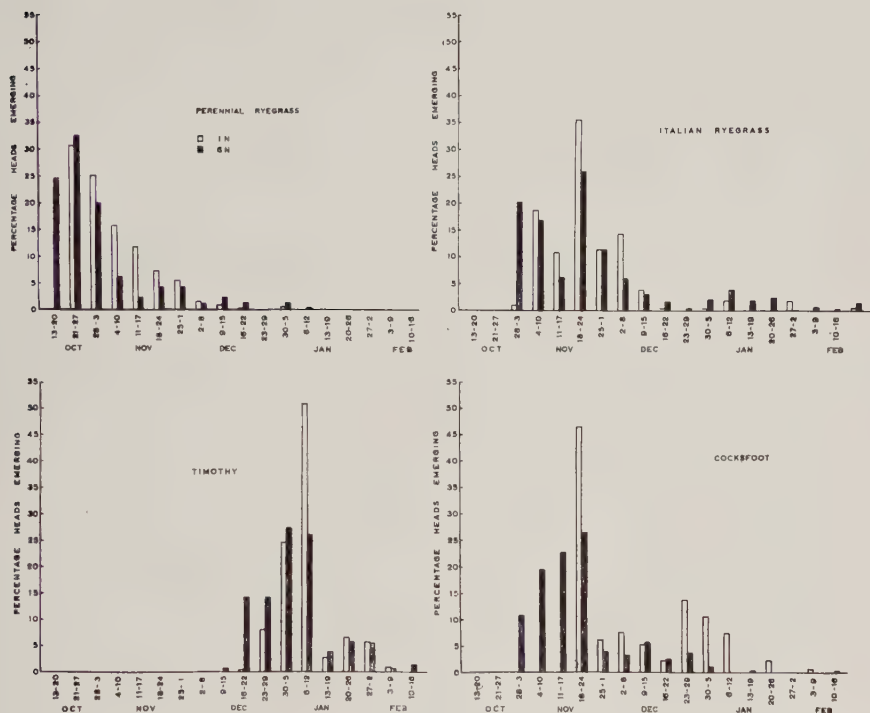


Fig. 6.—Percentages of heads emerging per week for each species and nitrogen level (expressed as percentages of the total number of headed tillers for each treatment).

Italian Ryegrass. Emergence was irregular under both nitrogen levels, most heads emerging between October and December.

Timothy. Under the high nitrogen level, 82% of the heads emerged in the four-week period beginning 16 December. A definite peak was obtained for the low nitrogen level, 75% of the heads emerging in the two weeks after 30 December.

Cocksfoot. Under the high nitrogen level, 79% of the heads emerged during the four-week period beginning 28 October. A very pronounced peak of head emergence is shown under the low nitrogen level for the week 18-24 November during which 48% of the heads emerged.

Effect of High Temperature on Heading of Perennial Ryegrass

A comparison of the number of heads of perennial ryegrass produced under controlled high and moderate temperature conditions, under a 15-hour daylight is given in Table 6.

There was an almost complete absence of heading in the high temperature treatment.

TABLE 6. NUMBER OF HEADS OF PERENNIAL RYEGRASS EMERGED UNDER CONTROLLED HIGH AND MODERATE TEMPERATURES (per 10 plants)

No. of Heads Formed Between 24 Jan.-4 March, 1959	
90°/70°F	70°/50°F
4	147

DISCUSSION

The results of this study must be considered in the light of the experimental techniques used. In particular it should be noted that the plants were transferred to beds in October-November during reproduction, and also that the replacement plants were divided in May and again in September-October.

No floral initiation was observed during the winter; the main periods of floral initiation were: perennial ryegrass late September; cocksfoot early October; Italian ryegrass mid October; and timothy mid November. In perennial ryegrass, Italian ryegrass, and timothy, tillers originating as far apart as May and September gave a range in both time of floral initiation and ear emergence of only about two weeks. Small differences in time of ear emergence from tillers of varied dates of origin, have also been observed for ryegrasses (Cooper and Saeed 1949) and timothy (Langer 1956).

Of particular interest is the almost complete absence of floral initiation in all species after late December, even though daylength (Fig. 2) at this time is approximately two hours longer than that prevailing during the main period of floral initiation. There would appear to be some other factors responsible for this failure in floral initiation. This conclusion has previously been reached by Langer (1956), Cooper (1958), and Cocks (1958) for timothy. As is shown in Table 6, a high day/night temperature of 90°/70°F with a favourable 15-hour daylength will cause a severe reduction in the number of heads produced by perennial ryegrass. Maximum day temperatures of this order were recorded during the experiment (Table 2) and may be largely responsible for the poor heading of perennial ryegrass in late summer. The inhibition of heading by high temperatures has been observed in timothy by Cooper (1958). Whether the effect of high temperature in the section of the present study carried out in the growth cabinets was inhibition of floral initiation or prevention of culm elongation was not examined. However, in the field trial only a few floral growing points were observed for cocksfoot and Italian ryegrass, and none for perennial ryegrass and timothy, at the final harvest in April of all tagged tillers which had not produced seed heads. This gives some evidence that under field conditions failure in floral initiation rather than inhibition of culm elongation is largely responsible for poor heading after December.

In cocksfoot, the failure to produce floral primordia by any of the tillers formed after 6 October is of note. Gardner and Loomis (1953)

state that this species requires short days together with or followed by low temperature, for floral induction. It is likely that tillers formed after 6 October do not receive a sufficient number of short, cold days (Fig. 2) at Palmerston North. This provides indirect evidence for the localisation of floral induction within the particular tillers which received winter treatment. By contrast, there was no evidence for localisation of the winter-induction stimulus in perennial ryegrass, tillers formed in early December being capable of floral initiation.

Intervals from floral initiation to ear emergence in perennial ryegrass and Italian ryegrass tillers were relatively constant at about 36-42 days for most dates of origin, although minima of 30 and 21 days were recorded for these two species respectively. An interval of 31-45 days has been reported for perennial ryegrass (Cooper 1952) and a minimum period of 20 days for Italian ryegrass (Cooper and Saeed 1949). In the present study, timothy and cocksfoot showed intervals decreasing from early- to late-formed tillers, to reach minima of 32 and 24 days respectively. A similar interval has been recorded under optimum photoperiod treatment for timothy (Evans and Allard 1934; Sprague 1948). Hanson and Sprague (1953) have reported intervals of 17 days for early, and 24 days for late plants, of cocksfoot.

The leaf number at ear emergence decreased with later dates of tiller origin to reach minima of 6 for perennial ryegrass, 7 for Italian ryegrass, 14 for timothy, and 9 for cocksfoot. Leaf numbers as low as 5, 4, and 11 were recorded for individual tillers of the first three species above. A decline in leaf number for later-formed tillers has also been reported for Wimmera ryegrass (Cooper and Saeed 1949) and timothy (Langer 1956). The lack of a minimum vegetative condition (Purvis and Gregory 1937; Sharman 1947; Cooper and Saeed 1949; Lamp 1952) may be largely responsible for the delay in floral initiation in tillers formed in late spring or early summer. However, two untagged tillers of perennial ryegrass were observed to head with the abnormally low leaf number of two. Cooper and Saeed (1949) have observed Wimmera ryegrass heading after the formation of the prophyll only. It would thus seem that the minimum vegetative requirement for floral initiation in ryegrasses is very small.

For perennial and Italian ryegrass the percentage of fertile tillers was high in those formed up to late spring. In timothy the percentage was likewise high for those formed before early spring, but tillers formed after early October showed a rapid decrease in fertility. In cocksfoot the percentage of fertile tillers was high only for those tillers originating before the end of June. The high percentage of vegetative tillers among those arising after late winter should allow an earlier recovery of vegetative production in cocksfoot than in the other three species, in which a large percentage of vegetative tillers was not noted until early November. In all species the percentage of dead tillers increased over the summer period, this being especially marked in Italian ryegrass.

Under the high nitrogen level, the dates of floral initiation and ear emergence for the winter and early spring tillers were earlier by a

week in perennial ryegrass and timothy and three weeks in cocksfoot. Italian ryegrass was two weeks earlier in initiation but only one week earlier in ear emergence. With the exception of timothy, the difference between high and low nitrogen levels was more marked in the late spring and summer tillers. High nitrogen increased the percentage of fertile tillers in all species, particularly of those tillers arising during the late spring and summer period. In cocksfoot, nitrogen was important in increasing the fertility of winter-formed tillers. High nitrogen also prolonged the period over which floral initiation and ear emergence occurred except in perennial ryegrass, where flowering was relatively short in both nitrogen treatments.

It is noticeable that the tillers with the highest ear production potential were those formed during winter and early spring in perennial ryegrass, Italian ryegrass, and timothy, and those formed during early winter in cocksfoot. Encouragement of these tillers would seem important for maximum seed-head production. These tillers do not differ much in their time of floral initiation and ear emergence. Thus, besides having a high heading potential, they will also be at much the same stage of development, and consequently most desirable tillers for seed production. These tillers will suffer a severe reduction in number if grazing is continued after floral initiation and when culm elongation is taking place. Under the conditions of this experiment grazing could have been continued until mid September for perennial ryegrass, and late September for Italian ryegrass. Tillers recovering from this grazing, or originating immediately afterwards, would be capable of producing a high percentage of seed heads. Cocksfoot and timothy, however, produced relatively few seed heads from tillers formed after mid July. Thus lenient grazing and early closing to allow these tillers to develop seed heads would seem advisable.

The 1958 spring temperatures were 2-3°F above the average and thus it is highly probable that the onset of the reproductive phase, reported here, was slightly different from that normal in this district.

For maintaining leaf production, a severe grazing after the main period of floral initiation in each species would be of appreciable value. A high proportion of the potential seed heads would thus be removed, and replaced by tillers which will largely remain vegetative. Consideration of the number of days between floral initiation and ear emergence may help in determining the grazing frequency necessary to control aftermath flowering. Management trials both for seed production and for continued vegetative growth could well be designed with these data as a basis.

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THE CONCENTRATION OF RADIOIODINE BY THE FOETAL THYROID GLAND AND ITS RELATION TO CONGENITAL GOITRE IN SHEEP

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(Received for publication, 13 May 1959)

Summary

The ability of the foetal sheep thyroid to accumulate radioiodine varies markedly during gestation. From sixty to one hundred days of foetal age the foetal and maternal glands are similar in this respect but from one hundred to a hundred and twenty days the foetal capacity increases until it is four times greater than the maternal and continues at this level until parturition.

The ingestion of kale by the ewe is more effective in producing congenital goitre during the fourth month of pregnancy than it is at other times. This corresponds to the period during which the foetal gland is developing its high power of absorption of iodine.

INTRODUCTION

When pregnant ewes are grazed on thousand-headed kale their lambs may be born with severe, and often fatal, goitre (Sinclair and Andrews 1954). Though the ewes themselves show no clinical signs, the use of radioiodine shows their thyroid function to be disturbed (Wright and Sinclair 1958).

This goitre may be prevented by treatment with iodine, and the practical importance of the time and method of such prophylaxis (Sinclair and Andrews 1958) suggested an investigation of the relative powers of the maternal and foetal glands to concentrate radioiodine at various stages of pregnancy.

Several reports have appeared showing that the placenta concentrates radioiodine in such a way that the foetal plasma may contain up to four times as much as the maternal (Hall and Myant 1956; Logothetopoulos and Scott 1956; Nataf *et al.* 1957), and Geloso (1956) found that the foetal rat thyroid concentrated radioiodine to a greater extent than did the maternal gland. At the time of our experiment in 1957 this had not been extended to the sheep nor had a complete description of the relationship between the maternal and foetal glands during the course of pregnancy been obtained. Since that time a report on sheep has appeared (Barnes *et al.* 1958). This work differs from ours in that it describes the effect of regular administration of radioiodine as opposed to our single dosage. The similarities and difference between the two sets of results are discussed below.

In the first part of this paper we describe experiments, which were carried out at Wallaceville in 1957, and which compare the foetal and maternal thyroidal accumulation of radioiodine at various changes of

gestation. The results of this work suggested the experiment on congenital goitre at Manutuke in 1958, which is reported in the second part.

EXPERIMENTAL METHODS

The Concentration of Radioiodine

Ten ewes at varying stages of pregnancy were given $7 \mu\text{C } ^{131}\text{I}$ intraperitoneally and killed 72 hours later.

The thyroid glands of both the ewe and the foetus were removed and, after mincing in a micro-mincer, their radioactivity was measured by counting approximately equal amounts of the minced tissues in 10 ml water in the phantom sheep thyroid described elsewhere (Wright 1958, 1959).

Congenital Goitre

Five groups of five pregnant ewes were allowed to graze on thousand-headed kale for one or two monthly periods. For the rest of the gestation period they ran on pasture with the sixth (control) group.

All lambs were killed soon after birth and their thyroid glands removed and weighed.

RESULTS AND DISCUSSION

The Concentration of Radioiodine

The radioactivity per gramme of foetal thyroid tissue was divided by that of maternal tissue and this ratio is plotted against foetal age in Fig. 1.

The type of curve we expected to obtain was one in which the ratio was very low until pregnancy was well advanced. When the

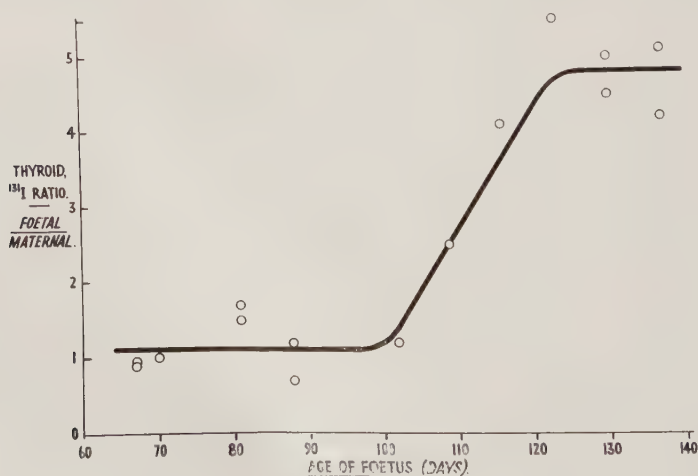


Fig. 1.—The change in the absorption of radioiodine by the foetal thyroid relative to the maternal with foetal age.

thyroid began to function, this ratio would rise steadily and reach a value greater than one, at parturition or just before.

Instead of this we found that the ratio was approximately one, even at the earliest measurements, and that during the fourth month it rose steadily to a value between four and five, and remained there until birth.

As already mentioned, Barnes *et al.* (1958) carried out similar experiments involving daily dosing with radioiodine. They used two levels of 1.5 μc and 5 μc of radioiodine daily. To compare their results with ours, ratios of foetal to maternal radioactivity have been calculated from their 1.5 μc experiment and these are shown in Table 1 with corresponding values read from the graph of Fig. 1.

TABLE 1. COMPARISON OF RESULTS OF BARNES *et al.* WITH THOSE OF WRIGHT AND SINCLAIR

	Foetal $^{131}\text{I}/\text{g}$ thyroid Maternal $^{131}\text{I}/\text{g}$ thyroid				
	Days Pregnant				
	70	85	100	115	130
Barnes <i>et al.</i>	0.2	0.3	0.7	1.5	2.5
Wright and Sinclair	1.2	1.2	1.2	3.0	4.6

These two sets of results really measure different things. In that of Barnes *et al.* the constant intake of radioiodine will have resulted in near equilibrium conditions and the figures will be an estimate of relative storage by the glands, whereas, in our experiment, the single dose procedure will give an indication of rate of turnover. It can be seen that, while the two sets of figures agree as to the great increase in activity about the fourth month, the first set show a more gradual change, as would be expected, while our figures show a much more abrupt transition. This difference might be explained by postulating that the foetal thyroid has the same iodine-trapping ability as the maternal gland from an early stage but has a much inferior binding capacity. The latter develops gradually with the growth of the lamb and then at the fourth month the placenta develops a special ability to trap iodine. An experiment designed to test this hypothesis would be valuable.

Congenital Goitre

The results from the experiment reported above suggested that the fourth month might be the critical period for congenital goitre. We already knew that it could be prevented by two doses of iodine, one at the beginning of the fourth month and one at the beginning of the fifth, but that a single dose at the latter time was less effective. (Sinclair and Andrews 1958). We therefore ran an experiment in 1958 to test the goitrogenic effect of grazing on kale for different

periods during pregnancy. Details of the grazing periods and the thyroid weights produced in the lambs are given in Table 2. Individual figures are given on account of the difference in distribution between groups 4 and 5 on the one hand, and groups 1, 2, 3, and 6 on the other. It appears that the individuals of 4 and 5 fall into two classes; not affected (or barely affected); and markedly affected—without intervening gradations, whereas the controls and group 6 are both fairly homogeneous.

TABLE 2. WEIGHTS OF THYROIDS OF LAMBS FROM EWES GRAZING ON KALE FOR VARIOUS PERIODS

Group	Period on Kale	Lamb Thyroid Weights							
		Ewe 1	Ewe 2	Ewe 3	Ewe 4	Ewe 5	Mean	±	S.E.
1	Nil	1.3 1.2	1.2	0.8 0.9	1.0 0.9	0.8 0.9	1.0	±	0.1
2	30-90 days	1.3	1.0	1.5	1.6	0.9 0.9	1.2	±	0.1
3	60-90 days	1.3	0.9	1.2 1.3	2.5 ¹	1.0	1.1	±	0.1
4	90-120 days	4.6 4.4	1.1 1.1	3.1 4.2	1.6 1.3	1.1	2.5	±	0.5
5	90-142 days	2.3 3.6	4.6 0.8	1.0	7.7	— ²	3.2	±	1.1
6	120-142 days	1.4	1.7	1.9	1.5	— ³	1.6	±	0.1

¹ Born dead. Head swollen. Not included in mean.

² Born dead. Head grossly swollen.

³ Ewe died. Foetus putrefied.

This heterogeneity in the 4th and 5th groups could be due to a threshold effect in secretion of the goitrogen whereby the unaffected lambs would be from dams which had gained control of the blood level of the goitrogen by excretion. Such an effect has been observed with thiocyanate (Wright 1959). In general, it has been confirmed that the fourth month is the critical period, though grazing on kale during the fifth month produced a highly significant increase in the size of the thyroids. The increase in group 5, following the ingestion of kale during the fifth month, over that, in group 4, caused by grazing kale in the fourth month alone is not significant. There was no fatal goitre at Manutuke in the 1958 season, and the heaviest of the glands listed in Table 2 is 7.7 g, compared with 25.0 g in 1953 (Sinclair and Andrews 1954) and the astonishing weight of 88 g in 1956 (Sinclair and Andrews 1958).

CONCLUSIONS

This work shows that the foetal thyroid gland can collect iodine to an extent equal to that of the maternal gland from as early as 60 days. This ability develops greatly during the fourth month of pregnancy to a level four times as high. It is at this stage that the developing lamb is most vulnerable to the effect of the goitrogen found in thousand-headed kale.

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CHERRY VARIEGATION IN NEW ZEALAND

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(Received for publication, 30 April 1959)

Summary

Cherry variegation, a non-infectious disease probably arising from bud sports, is widespread in New Zealand.

The most characteristic symptom is a white spotting or streaking of leaves sometimes accompanied by distortion or upward rolling of leaf margins. Severely affected trees are stunted and bear small fruits.

It has been found on four varieties of sweet cherry and on one Oriental flowering cherry variety.

The disease is perpetuated on scions and may appear on the progeny of affected trees.

It is considered to be the same as the disease known as variegation, or virus-like variegation, in Europe, and as cherry white crinkle in North America.

INTRODUCTION

A white or creamy-white spotting and streaking of leaves on several varieties of sweet cherry (*Prunus avium* L.) has been known in New Zealand for a number of years. It was originally recorded under the name of cherry marbling as a virus disease on the variety St. Margaret's (Helson 1953), but in the following year its virus nature was disclaimed (Anon. 1954). The disease was termed "a mosaic disease of cherries" and its nature as a non-infectious variegation suggested (Chamberlain, 1954a), and it was also recorded as a virus-like disease (Chamberlain 1954b).

Symptoms similar to those occurring in New Zealand were observed on cherries at East Malling Research Station during a visit by one of the authors in 1954. The condition appears to be the same as a non-parasitic disorder known as cherry white crinkle in North America (Dr. A. F. Posnette pers. comm.). Basing his opinion on photographs Dr. M. F. Welsh (pers. comm.) considered that the New Zealand disease and cherry white crinkle were identical.

Although known to occur in North America it does not appear to have been recorded there, but what appears to be the same disease has been recorded as variegation in Bulgaria (Atanasoff 1935; Christoff 1938), England (Posnette 1954), France (Bovey 1954), and Germany (Schuch 1957), and as a virus-like variegation in Italy (Picco and Scaramuzzi 1957).

As it appears to be identical with cherry variegation recorded from Europe, the New Zealand disease has been given this name.

SYMPTOMS

On young leaves, symptoms first appear as light green, irregular spots, streaks, or blotches. As leaves mature, the chlorotic areas



Fig. 1.—Cherry variegation on St. Margaret's sweet cherry. Leaves showing well distributed white pattern and slight distortion.
 $\times \frac{3}{4}$ Photo: S. A. Rumsey

become lighter in colour until they are creamy white or white (Figs. 1-3). Frequently there is a variation in degree of chlorosis, some areas being pale green and others almost white (Figs. 2 and 3a.). Sometimes chlorotic areas are arranged in a manner similar to the pattern in some types of marble, giving leaves a marbled appearance (Figs. 1 and 2). Symptom intensity varies from a few small scattered chlorotic spots per leaf (Fig. 3a) to an evenly distributed chlorotic pattern involving a high proportion of the leaf surface (Fig. 1). Leaves are sometimes distorted (Figs. 1 and 3b) and when symptoms are severe they often roll upwards at the margins (Fig. 2).



Fig. 2.—Cherry variegation on Bigarreau de Mezel sweet cherry. Leaves showing rolling of margins. Leaf on right shows light green as well as white areas.
Nat. size

Photo: S. A. Rumsey

On some trees only a few leaves are affected, while on others most leaves show chlorosis. Symptoms are sometimes confined to a single branch. Severely affected trees are smaller than normal, lack vigour, and bear undersized fruit.

VARIETAL RANGE AND INCIDENCE

Cherry variegation has been observed in New Zealand on the sweet cherry varieties Bigarreau de Mezel, Doncaster, Florence, and St. Margaret's. It has also been found on two unnamed varieties of



Fig. 3.—Cherry variegation on sweet cherry.

a. Leaf of Bigarreau de Mezel showing mild symptoms. Note both pale green and white areas.

b. Leaf from St. Margaret's seedling showing white areas accompanied by marked distortion.

$\times \frac{2}{3}$

Photos: S. A. Rumsey

sweet cherry and severe symptoms have been seen on a single tree used as a source of stocks for ornamental cherries.

The disease appears to be most common on the variety St. Margaret's, affected trees having been seen in many orchards. On this variety incidence in different orchards ranges from a few leaves on a single tree to marked symptoms on over 20% of all trees. The highest incidence observed, however, occurs in the variety Bigarreau de Mezel where in a block of 40 trees 38 are affected. The disease has been seen on three trees of Florence and on one of Doncaster. It has been found in Central Otago, Canterbury, Marlborough, and Manawatu, and probably occurs in all districts where cherries are grown.

ECONOMIC IMPORTANCE

Mildly affected trees do not show any marked reduction in vigour but there is undoubtedly some loss of crop. When symptoms are severe, trees are stunted and fruit fails to size up with the result that much of it is unsaleable. In the case of Bigarreau de Mezel mentioned above, the grower estimated that crop losses on severely affected trees ranged from 33% to 50%. Therefore, although over-all losses

TABLE 1. ATTEMPTED TRANSMISSION OF CHERRY VARIEGATION TO
Prunus SPECIES

Species Inoculated	Source of Inoculum	Duration of Experiment	No. Trees Inoculated	No. Trees Showing Symptoms
Sweet cherry (<i>Prunus avium</i> L.)	Sweet cherry	3 seasons	17	0
"	Sweet cherry	4 seasons	3	0
"	Sweet cherry	3 seasons	4	0
"	Sweet cherry	2 seasons	20	0
Mazzard cherry (<i>P. avium</i> L.)	Oriental flowering cherry (<i>P. serrulata</i> Lindl.)	3 seasons	10	0
"	Sweet cherry	4 seasons	3	0
Sour cherry (<i>P. cerasus</i> L.)	Sweet cherry	2 seasons	2	0
Cherry plum (<i>P. cerasifera</i> Ehr.)	Sweet cherry	2 seasons	6	0
"	Sweet cherry	2 seasons	3	0
"	Sweet cherry	1 season	3	0
Peach (<i>P. persicae</i> L.)	Sweet cherry	2 seasons	2	0
"	Sweet cherry	1 season	3	0
"	Sweet cherry	2 seasons	20	0
"	Oriental flowering cherry	3 seasons	2	0
Apricot (<i>P. armeniaca</i>)	Sweet cherry	1 season	3	0
"	Sweet cherry	4 seasons	6	0

are not serious, individual growers may suffer substantial crop reduction in some varieties.

TRANSMISSION EXPERIMENTS

Experiments at this station have shown that cherry variegation is consistently perpetuated in buds from affected trees. However, all attempts to transmit it by budding to sweet cherry, Mazzard cherry, peach and apricot seedlings, and to cherry plum stocks raised from cuttings, have failed (see Table 1). We have some evidence to suggest that, like sweet cherry crinkle leaf (Kinman *et al.* 1951), the condition may develop on seedlings raised from seed of affected trees. Of approximately 150 St. Margaret's sweet-cherry seedlings raised from seed secured from a block in which the disease is known to occur, three developed definite symptoms (Fig. 3b).

The available evidence suggests that cherry variegation is non-parasitic and probably arises from bud sports (mutations).

CONTROL

As cherry variegation is perpetuated in scions it is essential that all scion material should be secured from trees free from this disease. No information is available concerning the influence of affected stocks on scion growth but it would be advisable to propagate stocks from disease-free trees. Likewise seed for raising seedling stocks should be obtained from trees free from variegation.

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NOTE ON A MODIFICATION IN TECHNIQUE FOR INACTIVATING APPLE MOSAIC VIRUS IN APPLE WOOD BY HEAT TREATMENT

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(Received for publication, 2 July 1959)

Since Kunkel (1935) established that exposure of virus-infected trees in pots to temperatures ranging between 34.4° and 36.3°C was effective in freeing peach trees of either peach yellows or peach rosette, heat treatment for the inactivation of different viruses in plants has become established practice.

Unsuccessful attempts to inactivate apple mosaic in scion-wood by heating at 36°, 48°, and 50°C have been reported (Thomas 1937; Louw 1944). Posnette and Cropley (1956) infected apple seedlings with a virulent strain of this virus, by chip budding, and a year later when the seedlings were showing severe symptoms, placed them in a glass cabinet held at 37°C. Following removal in batches after 15, 27, and 35 days, the trees were cut back and planted in a nursery. A number of the heat-treated trees were symptomless in the following year, but a few of these developed symptoms in the second year.

The same authors (1958) postulated the survival of active virus only in the deep-seated tissue in the stele of some heat-treated plants and a subsequent slow translocation to leaves. Kunkel (*loc. cit.*) drew attention to the difficulty of destroying viruses in living roots embedded in moist soil, where evaporation could reduce the temperature below that of the tops. Kassanis (1954) also reported the development of symptoms when some heat-treated plants were returned to ordinary glasshouse conditions at about 20°C. He accepted Kunkel's explanation of the retention of virus in roots as a possible explanation of his results. However, he also reported that the development of symptoms on some cuttings taken from heat-treated plants indicated an erratic distribution of surviving virus in aerial parts as well.

Consideration of some of these disadvantages in treating wholly infected plants suggested the possibility of treating a small piece of infected material in the form of a bud shield inserted into a healthy seedling, and subjecting the budded plants to heat therapy as soon after bud insertion as was consistent with bud survival. This note records the results of such procedure.

Healthy Jonathan apple seedlings were raised in four-inch pots under glass. In mid December, 22 of these plants were prepared for treatment by working each with a single bud from a tree carrying a mild strain of apple mosaic; another group of 22 was budded with a moderate strain, while a third group of 22 was budded with a severe strain. For each group, infected buds were taken from one Jonathan tree carrying the appropriate strain. After budding, all 66 plants were held under glass for 26 days to allow bud union to take place. Then six seedlings from each group were put aside as controls, and the remaining plants were put into a heated glass cabinet held at $37^{\circ} \pm 1^{\circ}\text{C}$.

Successive batches were removed after 28, 30, 36, and 40 days. Three plants died during treatment, one from the mild and two from the severe group. After allowing a week for recovery under glass, all surviving treated trees and checks were planted in nursery rows in the open. Seedlings were cut back in September, and in the following growing season both stock and scion shoots were encouraged.

No symptoms developed on any of the heat-treated trees during the following summer but all check trees showed symptoms typical of the strain with which they had been inoculated.

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² Fruit Research Division, Department of Scientific and Industrial Research.

Although it is too early to be certain that apple mosaic has been eliminated from seedlings budded 26 days before heat treatment, it is clear that the newly inserted buds will survive heat treatment. If Posnette and Cropley's suggestion that apple mosaic can survive in deep seated tissues is correct, then treatment soon after budding offers a better chance of elimination than heating fully infected trees.

Since the initial experiment was made it has been found that a large percentage of apple buds will unite and survive when heat treatment starts 10 days after budding. In our most recent trials it seems that some buds of certain varieties, e.g. standard Delicious and the Malling rootstock XVI, will survive even when inserted only half an hour before the commencement of heat treatment. Thus unless virus movement in an apple seedling is extraordinarily rapid there appears to be a good chance of eliminating apple mosaic from many varieties. This modified technique has the added advantage of speeding up this type of experiment by a full growing season.

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SEED DEVELOPMENT IN RYEGRASS, AND IN RED AND WHITE CLOVER

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(Received for publication, 12 June 1959)

Summary

Studies were made of the rate of development of the seeds of perennial and Italian ryegrass, and of red and white clover.

Three developmental phases can be recognised; the first a period of rapid growth and constant high moisture percentage, lasting about 10 days from pollination, during which viability is nil; a second phase of 10–14 days, when growth is slower and percentage moisture falls steadily, with sudden acquisition of viability; and a final phase of 3–7 days in which dry weight is constant but moisture decreases markedly.

The effect of harvesting during these three phases is discussed, and mention is made of features useful in identifying the developmental stages.

INTRODUCTION

In harvesting seed, the stage at which the crop is cut affects both the yield and quality of the seed, and the ease with which it is cured and threshed. For most of our pasture species the optimum stage for cutting the seed crop is not known. To help answer this question the course of seed development of four common pasture species was examined in detail.

Similar studies of seed maturity, as it affects germination, have been made before, but the emphasis has been on cereal crops. Most attention has been paid to two main phases of seed development; the relationship of moisture content to dry weight, and the development of viable seed from time of anthesis. The moisture content of seeds of small grains at the time of near-maximum dry weight has been reported by Harlan and Pope (1923) as 42% in barley, by Burnett and Bakke (1930) as 40% in wheat, oats, and barley, and by Grabe (1956) as 47% in brome grass (*Bromus inermis*). Harlan and Pope (1922) state that certain barley varieties produce viable seeds as early as six days after pollination, while Bartel (1941), Hatcher and Purvis (1945) and Nutman (1941) report periods for various cereals varying from five to eight days. In brome grass, Grabe (1956) found viable seeds five days from anthesis, while seeds harvested at 10 days germinated as well as mature seed; the ability to germinate and the vigour of the resulting seedlings were found to be directly proportional to the supply of food reserves in the seed.

Frey *et al.* (1958) found that, with oats, near-maximum dry weight was reached at 45% moisture content. Germination increased with

* Now deceased.

maturity up to 20-28 days post-anthesis, although the first germinations were obtained at only four days. Griffeth and Harrison (1954) found, with reed canary grass (*Phalaris arundinacea* L.), that seed harvested at 66% moisture showed no germination, but two days later seed harvested at 48% moisture gave 30% germination. After this, germination steadily increased as moisture content decreased.

Working with white clover (*Trifolium repens* L.) Hyde (1950) showed that the same overall pattern of development applied to this legume.

METHODS AND RESULTS

New Zealand Certified strains of the following species were studied: Perennial ryegrass (*Lolium perenne* L.), Italian ryegrass (*L. multiflorum* Lam.) white clover (*Trifolium repens* L.) and cowgrass (*T. pratense* L.).

The ryegrasses were grown as spaced plants in the field, the white clover in a dense, pure stand, and the red-clover plants were grown outdoors in pots.

Seeds, derived from pollination on known dates, were identified, and random samples of such seeds were taken at intervals over the period of development and ripening. The weight of the seed was determined while fresh, and again after drying to constant weight at 105°C. Sub-samples were air dried and subsequently tested for germination.

Perennial Ryegrass

To identify flowers pollinated on a certain day, a piece of coloured wool was tied round the rachis at the time of flowering in such a position that the bottom two florets in each of the first two spikelets above the wool were all open at the time of marking. About 2,000 heads on 400 plants were marked in this way over a period of three days, wool of a different colour being used each day.

Two samples, each of 25 heads, were taken at intervals (one to three days) from the tagged plants, to give 100 dated seeds for each determination. The caryopses of one sample were weighed while fresh,

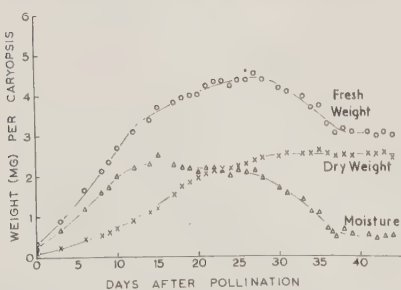


Fig. 1

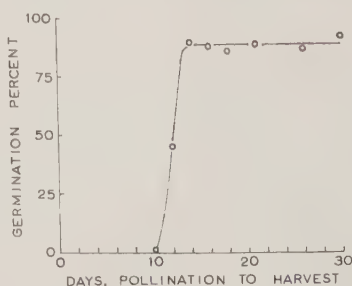


Fig. 2

Fig. 1.—Weight changes in the perennial ryegrass caryopsis during development.

Fig. 2.—Effect of stage of development at harvesting on the germinating capacity of perennial ryegrass seed.

and again after drying to constant weight at 105°C. The other sample of heads was air dried, and after four months storage the 100 identified seeds were tested for germination.

The results are shown in Figs. 1 and 2. Full germination capacity was reached by the fourteenth day from anthesis, when moisture content was 65% and the dry weight of the caryopsis was 44% of its final value.

The colour change in the pericarp, from green to yellow or purple, commenced at about the twentieth day, when dry weight of the caryopsis was about 75% of its final weight and moisture content 54% of the fresh weight; the glumes began to turn brown at the same stage of development. Full dry weight was reached at about 28 days, when the moisture content was 44%.

Italian Ryegrass

The method for this species was the same as that used for perennial ryegrass, and the results are shown in Figs. 3 and 4.

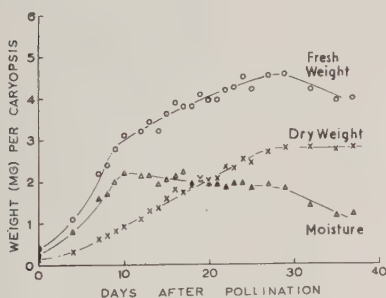


Fig. 3

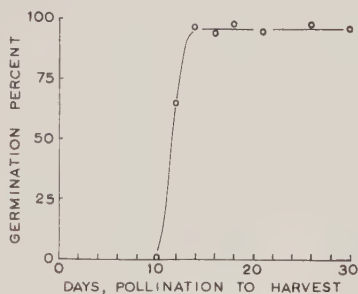


Fig. 4

Fig. 3.—Weight changes in the Italian ryegrass caryopsis during development.

Fig. 4.—Effect of stage of development at harvesting on the germinating capacity of Italian ryegrass seed.

Full germination capacity was reached by the fourteenth day, when the moisture content was 59%, and the dry weight of the caryopsis 48% of its final value. The colour of the pericarp and glumes began to change at the eighteenth day, when moisture content was 51% and the caryopsis 65% of final weight.

Maximum fresh weight was attained at 28 days, with a moisture content of 38%.

White Clover

Heads were marked by tying a piece of wool round the clover inflorescence between the reflexed and the open florets. Those florets above the tie were worked by bees on the date of marking, and by the following day were reflexed, the corollas brown, and no longer worked by bees. About 2,000 heads were marked in one day.

Every second day thereafter two samples, each of 25 marked heads, were harvested at random, one for the determination of fresh and dry weight and the other for germination studies. Only seeds from the whorl of pods immediately above the wool were taken into account.

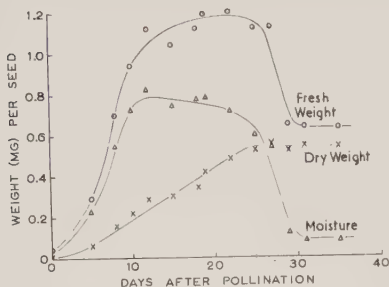


Fig. 5

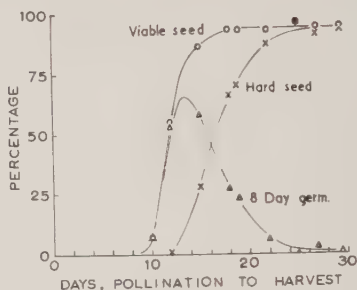


Fig. 6

Fig. 5.—Weight changes in the white clover seed during development.

Fig. 6.—Effect of stage of development at harvesting on some characteristics of white clover seed.

The results are shown in Figs. 5 and 6. Viability was acquired about the twelfth day, when moisture content was 77% and the dry weight of the seed 44% of its final weight. Colour began to change from green to yellow on the eighteenth day.

A drop in percentage germination in eight days occurred approximately two weeks after pollination, indicating the development of hard seeds.

Red Clover

Forty-eight plants were placed in a cage to prevent accidental pollination. When flowering took place, heads in bloom were marked with wool and sixty bumble bees (*Bombus* spp.) were placed in the cage for 24 hours. It was assumed that seed in the marked heads resulted from pollination during this period. As a check on the control of pollination a number of flower heads were protected from the bees by gauze bags. These heads produced no seeds.

Two samples, each of 25 marked heads, were taken every 2–3 days. From one sample the seeds were dissected out and their fresh and dry weights determined, while the second sample was air dried and stored for four months. The seeds were then dissected out and tested for germination.

The results are shown in Figs. 7 and 8. Seeds harvested 14 days after pollination were all viable, whereas, of that harvested four days earlier, only 2% germinated. Thus viability was acquired about the twelfth day, when moisture content was 74% and the dry weight was 43% of the weight of the mature seed.

Initially, the red-clover seeds were light green in colour, but when fully developed were yellow, or coloured with red or purple pigments.

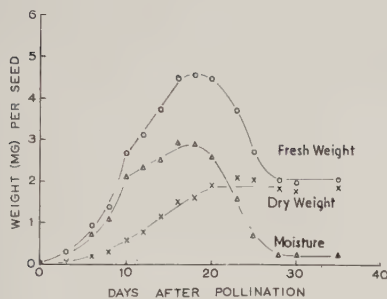


Fig. 7

Fig. 7.—Weight changes in red clover seed during development.

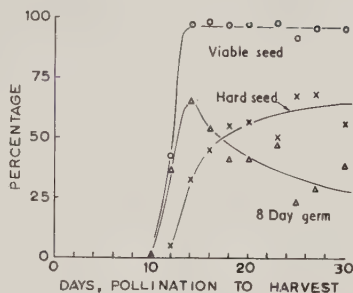


Fig. 8

Fig. 8.—Effect of stage of development at harvesting on some characteristics of red clover seed.

These colour changes commenced about the eighteenth day, when the seed was about four-fifths of its final dry weight, and were complete by about the twenty-fourth day.

As with white clover, a similar but less marked drop in percentage germination at eight days also occurred in red clover about two weeks after pollination.

DISCUSSION

In the development of both grass and clover seeds there are common features, and three developmental phases can be recognised in each case.

The first phase lasts for about ten days. During this period the rate of growth is rapid, and moisture percentage remains constant in the range of 75–80%. Seed harvested during this stage is not viable.

The second phase lasts for ten to fourteen days. There is a slower increase in dry weight, reaching a maximum at the end of the phase. The amount of water in the seed changes very little, but the percentage of water falls steadily. Seed becomes viable early in this phase.

In all species tested, viability is acquired with great rapidity, a period of four days only being required between the first viable seeds and full viability. Colour changes are an indication of approaching maturity, which gradually takes place during the latter half of this phase. In the legumes the development of hard seed begins somewhat later, and this brings about a decline in the germination percentage.

The third phase lasts three to seven days. Moisture content falls from about 40% to equilibrium, while dry weight remains approximately constant.

It is emphasised that the data for each species above were collected in different years. Further, the weather in Palmerston North may be far from stable during a particular harvest period. As the effects of weather and of seasonal differences on speed and uniformity of seed

development are unknown, comparisons between the species, or with overseas results, in their rates of development of full viability must take this into account. In particular, although all the species in this study developed viability at about twelve days from pollination, this result should be treated with some caution.

Hyde (1950) listed three important aspects of seed quality as viability, seedling vigour, and storage life. Viability is acquired at about twelve days from pollination, but at the time when translocation of food materials to the seed is only just commencing. Work with white clover indicated that vigour of seedlings from immature seeds suffers because of inadequate seed reserves. Seed harvested at the end of the first stage would possess viability, but not seedling vigour. This vigour is not gained until the end of the second phase.

Similarly, immature seed has been shown to deteriorate more rapidly in storage than mature seed, and is one of the main factors in reducing the quality of commercial seed. In all species studied, viable seeds may be harvested twelve to fourteen days after pollination, but since the seed weight at this stage is only about one third of mature weight, all such seed would be removed in commercial cleaning.

It would appear advisable, then, from the viewpoint of quality of the seed crop, to harvest as late as possible up to full maturity, certainly no earlier than the commencement of the third phase of development. Even then, owing to high respiration rate and 40% moisture content, care would be necessary to prevent overheating of the harvested crop.

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THE USE OF DIPHENYLAMINE AND OTHER CHEMICALS TO CONTROL SUPERFICIAL SCALD OF APPLES

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(Received for publication, 3 June 1959)

Summary

Dips of diphenylamine 1,000 p.p.m. in aqueous alcoholic solution controlled superficial scald on 11 varieties of apples, but not on Statesman.

A similar formulation applied as a spray to fruit in cases was equally successful, but some damage occurred to the bottom fruit where drainage was incomplete.

Paper wraps containing DPA completely controlled scald in Granny Smith apples. Oiled wraps did not give adequate control of scald in immature, early picked fruit.

Phenothiazine 1,000 and 2,000 p.p.m. did not control scald and caused damage to some varieties. Several other unsuccessful materials are noted.

Diphenylamine does not aggravate other storage disorders, and there is some evidence that it may reduce both internal breakdown and core flush.

INTRODUCTION

In common with apples grown in other countries, New Zealand varieties are subject to superficial scald (Padfield 1954). The most efficient and widely adopted method of control of this disorder has been the use of oiled wraps (Brooks *et al.* 1919). This method has never given complete control, particularly over long storage periods, and many other suggestions for increasing the efficiency of scald control have been tried, but none has been adopted (Padfield 1958).

Smock (pers. comm. and 1957) reported successful control of scald in apples using diphenylamine (DPA) either as a post-harvest dip at 1,000 p.p.m. or when incorporated in wraps. A series of experiments has been conducted in the three seasons 1956 to 1958, to test the efficacy of DPA and other chemicals for control of superficial scald in New Zealand-grown apple varieties.

MATERIALS AND EXPERIMENTAL PROCEDURE

In experiments conducted in 1956 and 1957, apples were dipped in solutions of DPA in 50% ethyl alcohol. No wetting or spreading agent was used. Fruit was simply dipped in such solutions and allowed to drain before being returned to the cases. In 1958, suspensions were prepared by dissolving DPA in isopropyl alcohol and stirring this solution into water immediately before use. The final strength of isopropyl alcohol was 5% with 1,000 p.p.m. DPA, 2.5% with 500 p.p.m. DPA, etc. 0.075% Tween 20 was incorporated as wetting agent. Analytical

grade DPA was used in 1956, 1957, and early 1958; for the remainder of the work commercial flake DPA was used.

A small stock of hand-made wraps prepared by dipping plain sulphite wraps in 1,000 p.p.m. DPA was used in 1956. Commercially manufactured wraps* reputedly containing 0.75–2.0 mg of DPA per 100 sq. in. of wrap were used in 1957. Australian wraps reputedly containing 1.8 mg per wrap were also used in 1957 and 1958. However, analyses for DPA content of these wraps made by the method of Harvey (1958) revealed values of 0.15–0.45 mg for the American wraps and 1.05 mg for the Australian wraps.

In 1956, most of the main apple varieties known to develop scald were included in the experiments, but in this and later years most of the work was carried out using Granny Smith, the most scald-susceptible variety grown in New Zealand. Unless specifically stated, samples of each variety were taken from commercial lines and harvested at export maturity. These samples were dipped, sprayed or wrapped, held at air temperature for 7 days, and then cool-stored at the appropriate temperature.

In 1958, DPA was applied by spraying fruit from four Hastings orchards *in situ* in bushel cases. Sufficient solution to wet every apple with either DPA solution or water as control was applied by knapsack sprayer to the top of each case, and allowed to drain out from the bottom. A trial application indicated that approximately one litre per bushel was required for complete wetting.

Throughout the series of trials, DPA-treated samples were always compared with untreated fruit, and, in some experiments, with fruit dipped in 50% alcohol, or wrapped in commercial oiled wraps (13.9% oil).

During the storage period, small test samples of untreated fruit were removed at monthly intervals and held at between 60° and 70°F for 14 days before being examined for scald. When approximately 50% of such apples showed scald, the main treatment samples were removed and examined after a similar holding period. Samples stored during 1956 generally consisted of three bushels per treatment, fruit size in each sample being uniform or with only slight variation. In subsequent years samples mainly consisted of four-bushel units. In 1956, the varieties Dougherty, Frimley Beauty, Granny Smith, Rome Beauty, Statesman, and Sturmer were not dipped in DPA until a considerable time after harvest (Table 1).

The efficacy of phenothiazine, a chemical related structurally to diphenylamine, in controlling scald was tested in 1957 and 1958. This material was applied only by dipping fruit in solutions.

RESULTS

Scald Control in Apple Varieties

The results in the 1956 experiment (Table 1) show that, when compared with oiled wraps, DPA as a post-harvest dip gave comparable

* Supplied by United Paper Company, Tampa, Florida, U.S.A.

TABLE 1. INCIDENCE OF SUPERFICIAL SCALD IN UNTREATED, OIL-WRAPPED AND DIPHENYLAMINE-WRAPPED OR -DIPPED FRUIT, TOGETHER WITH DAMAGE DUE TO DIPHENYLAMINE TREATMENTS, 1956

All results expressed as percentages

Variety	Harvest Date	Treatment Date	Examination Date	Treatment								Damage due to Diphenylamine Treatments			
				Untreated		Commercial Oiled Wraps		Diphenylamine 1,000 p.p.m.							
				Superficial		Superficial		Superficial		Superficial		Superficial		Slight	Severe
				Slight	Severe	Slight	Severe	Slight	Severe	Slight	Severe	Slight	Severe		
Ballarat Delicious Dougherty Frimley Beauty Granny Smith (1st exam.) Wrap	30 Mar.	3 Apr.	9 Oct.	14	3	1	0								
	23 Mar.	29 Mar.	30 Oct.	6	2	1	0								
	2 May	23 May	30 Oct.	15	22	18	3								
	18 Apr.	22 May	7 Sept.	31	38	5	0						17		0
	29 Apr.	22 May	4 Oct.	38	38	8	1								
Granny Smith (2nd exam.) Wrap	29 Apr.	22 May	14 Nov.	16	80	17	11								
				2†	3†	7†	5†								
Red Statesman Rome Beauty 1 Rome Beauty 2 Statesman Sturmer	11 Apr.	13 Apr.	15 Oct.	11	54	9	46								
	17 Apr.	20 Apr.	18 Sep.	26	48										
	7 May	23 May	19 Sep.	7	3	2	0								0
	17 Apr.	22 May	24 Apr.	10	36	7	7						44		52
	17 Apr.	22 May	24 Apr.	29	43	32	22						21		9

† Senescent scald

TABLE 2. INCIDENCE OF SUPERFICIAL SCALD IN UNTREATED, ALCOHOL-DIPPED AND DIPHENYLAMINE-DIPPED FRUIT, 1957

All results expressed as percentages

Variety	Harvest Date	Treat- ment Date	Exam- ination Date	Treatment									
				Untreated		Alcohol 50%		Diphenylamine 500 p.p.m.		Diphenylamine 1,000 p.p.m.		Diphenylamine 2,000 p.p.m.	
				Superficial Scald Slight	Severe	Superficial Scald Slight	Severe	Superficial Scald Slight	Severe	Superficial Scald Slight	Severe	Superficial Scald Slight	Severe
Ballarat	28 Feb.	1 Mar.	25 June	21	52					0	0		
Cox's Orange Pippin	21 Feb.	21 Feb.	23 Apr.	3	1					0	0		
Delicious	11 Apr.	13 Apr.	20 Nov.	10	3					1	0		
Dougherty	6 May	8 May	6 Nov.	31	28					2	0		
Frimley Beauty	24 Apr.	26 Apr.	2 Sep.	17	5	13	5	0	0	0	0		
Kidd's Orange Red	25 Feb.	1 Mar.	7 May	19 +12	23 +12					0 +10	0 +10		
Red Statesman	14 Apr.	15 Apr.	23 Sep.	24	14					9	1	11	2
Rome Beauty	6 May	8 May	29 July	0	0	0	0	0	0	0	0		
Statesman	24 Apr.	26 Apr.	19 Sep.	3	1					14	1	19	2
Sturmer	8 Apr.	9 Apr.	11 Nov.	28	9	15	3					10	0

† Wrinkled scald.

and often superior scald control on most varieties. The limited wrapping treatment indicated that DPA was likely to be equally efficacious when incorporated in wraps. Virtually complete control of scald (Fig. 1) was obtained on the most susceptible variety, Granny Smith. When this variety was held a further 40 days in store it could be seen that DPA had no effect on senescent scald (Padfield 1954).

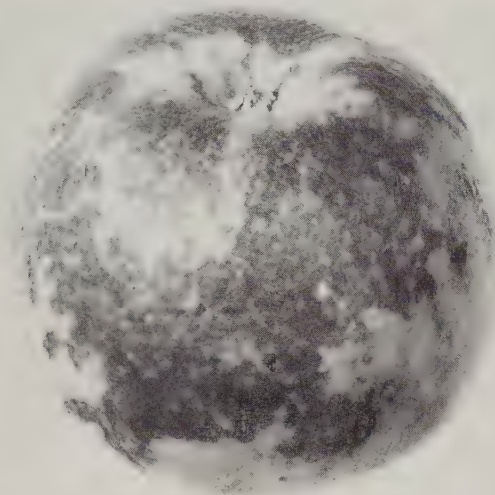


Fig. 1.—Characteristic blotchy symptoms of severe superficial scald on Granny Smith apples. Controlled by DPA.

Photo: J. Endt.

On one variety, Statesman, DPA apparently aggravated scald. In three varieties, Frimley Beauty, Rome Beauty (Fig. 2), and Sturmer, damage was caused either by alcohol or DPA or both. This took the form of black or dark-brown, slightly depressed spots located around lenticels. It was not as severe on Sturmer as on the other two varieties. A symptom similar to Jonathan spot developed on both Frimley (Fig. 3) and Rome Beauty. This was not controlled by DPA.

Similar results were obtained in a further experiment in 1957 (Table 2), when DPA at 1,000 p.p.m. controlled scald in a number of apple varieties. The anomalous result obtained with the variety Statesman was repeated, scald on this variety apparently increasing with application of DPA. As in the preceding year, DPA did not eliminate scald on Red Statesman and Sturmer, even at 2,000 p.p.m. At 500 p.p.m., scald on Frimley Beauty was eliminated. No spotting damage on Frimley Beauty, Rome Beauty, and Sturmer occurred in 1957. Scald was not controlled by aqueous alcoholic dips without DPA, substantiating a previous result (Padfield 1958).

The "wrinkled" scald symptom (Fig. 4) observed on Kidd's Orange Red was similar in appearance to "light induced" scald (Tiller, 1932; Padfield, 1954). This was not apparent on fruit at harvest and was thought to be a delayed symptom of this disorder,

TABLE 3. INCIDENCE OF SUPERFICIAL SCALD IN FRUIT IN VARIOUS CONCENTRATIONS OF DIPHENYLAMINE, 1958.

All results expressed as percentages

Variety	Harvest Date	Treatment Date	Examination Date	Treatment											
				Untreated		Diphenylamine 250 p.p.m. 1.25% Alcohol		Diphenylamine 500 p.p.m. 2.5% Alcohol		Diphenylamine 1,000 p.p.m. 5.0% Alcohol					
				Superficial Scald	Severe	Slight	Severe	Superficial Scald	Slight	Severe	Superficial Scald	Slight	Severe		
Ballarat	28 Feb.	4 Mar.	4 Aug.	45	24				*26		9				
Cox's Orange Red	17 Feb.	18 Feb.	5 May	22	6	*16		6	*7		1	*0.3		0	
Delicious	25 Mar.	25 Mar.	18 Nov.	11	19	2		0.3	1		0	0		0	
Dougherty	6 May	8 May	18 Nov.	1	1	Not examined as checks showed little scald									
Frimley Beauty	27 Apr.	28 Apr.	2 Sep.	5	2	1		0	3		0	4		1	
Golden Delicious	28 Mar.	28 Mar.	3 Sep.	0	0	0		0	0		0	0		0	
Kidd's Orange Red	20 Feb.	20 Feb.	7 May	30	25				*2		0	*0		0	
Red Statesman	23 Apr.	24 Apr.	23 Sep.	11	1	13		2	8		1	6		0	
Rome Beauty	28 Apr.	29 Apr.	8 Sep.	5	1	1		0	0.4		0	0		0	
Statesman	28 Apr.	29 Apr.	23 Sep.	20	3	14		0.3	10		0	6		0	
Sturmer	10 Apr.	12 Apr.	30 Oct.	32	13	0.2		0	0		0.2	1.5		0	

* 50% ethyl alcohol.

All others treated with isopropyl alcohol



Fig. 2.—Damage of Rome Beauty apples following treatment with DPA in alcohol. *Photo: J. Endt.*

In 1958, apples of a number of varieties were dipped in DPA at 250, 500, and 1,000 p.p.m. The early varieties (Ballarat, Cox's Pippin, Kidd's Orange Red) were treated with DPA in 50% ethyl alcohol, as all varieties had been in the previous two seasons. The late varieties in 1958, however, were dipped in suspensions of DPA prepared from concentrates in isopropyl alcohol.



Fig. 3.—“Jonathan spot” type symptom on Jonathan Beauty apples. Not controlled by DPA. *Photo: J. Endt.*



Fig. 4.—“Wrinkled scald” symptom in Kidd’s Orange Red apples.
Not controlled by DPA.

Photo: J. Endt.

A considerable degree of control was obtained using DPA at 500 p.p.m., but 1,000 p.p.m. was generally necessary to ensure complete control. It is interesting to note confirmation of the previous results with the variety Statesman.

TABLE 4. DAMAGE (%) FROM DIPHENYLAMINE DIPS AT VARIOUS CONCENTRATIONS, 1958

Variety	Concentration of Diphenylamine			
	0	250 p.p.m. 1.25% Alcohol	500 p.p.m. 2.5% Alcohol	1,000 p.p.m. 5.0% Alcohol
Delicious	0	0	0	32
Frimley Beauty	0	1	5	19
Golden Delicious	0	1	4	16
Rome Beauty	0	0	1	6

Diphenylamine: commercial grade
Alcohol: isopropyl

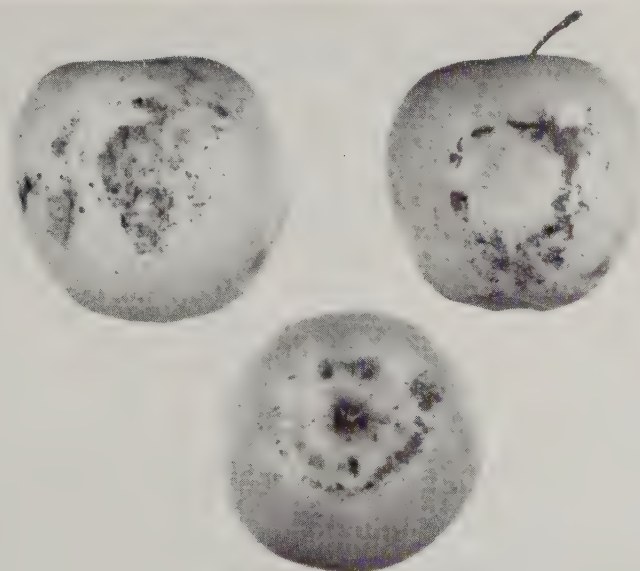


Fig. 5.—Damage to Golden Delicious following treatment with DPA in alcohol.

Photo: J. Endt.

TABLE 5. COMPARISON OF INCIDENCE OF SUPERFICIAL SCALD ON GRANNY SMITH APPLES IN PLAIN AND OILED WRAPS AND VARIOUS CONCENTRATIONS OF DIPHENYLAMINE IN WRAPS AND DIPS, 1957

Treatment	Number of Apples	Scald %	
Plain wraps	452	94	
Oiled wraps	452	19	
DPA 500 p.p.m. Dip	370	0.5	Harvest date 29 April
DPA 1,000 p.p.m. Dip + Tween 20 0.05%	344	0.3	Treatment date 30 April to 2 May
DPA 1,000 p.p.m. Dip	364	1	Examined 1 to 14 October
DPA 2,000 p.p.m. Dip	432	1	Quantity expressed per wrap of 100 sq. in.
*DPA wrap 0.75 mg	452	0.4	
*DPA wrap 1.5 mg	452	2	
*DPA wrap 1.5 mg + oil	449	0	
*DPA wrap 2.0 mg	452	0.4	

* Paper wraps supplied by United Paper Company, Tampa, Florida, U.S.A.

In four varieties damage occurred (Table 4). In Rome and Frimley Beauty this took the form of lenticel spotting similar to that seen in 1956 (Fig. 2). In Delicious and Golden Delicious, lesions were irregular and larger in area, dark-brown to black in colour, and slightly depressed (Fig. 5).

Scald Control in Granny Smith Apples

In 1957 and 1958, the apple variety most susceptible to scald, Granny Smith, was used to compare various methods of applying DPA. In 1957 almost complete control of scald was obtained with concentrations as low as 500 p.p.m. DPA dip and with 0.75 mg DPA per 100 sq. in. of wrap (Table 5).

No differences in scald control were apparent in fruit treated by dipping when this fruit was placed in store wet or when it was first dried (Table 6).

TABLE 6. METHOD OF APPLICATION OF 1,000 P.P.M. DIPHENYLAMINE IN 50% ETHYL ALCOHOL TO CONTROL SUPERFICIAL SCALD OF GRANNY SMITH APPLES, 1957

8 bushels per treatment

Picked 30 April

Treated 3 May

Examined 14 October

Treatment	Superficial Scald %	
	Slight	Severe
Check, water dip Fruit wet into store	11	82
Check, water dip Fruit dry into store	12	82
DPA 1,000 p.p.m. dip Fruit wet into store	1	0
DPA 1,000 p.p.m. dip Fruit dry into store	1	0.3
DPA 1,000 p.p.m. sprayed on fruit <i>in situ</i> in case. Fruit dry into store	1	1

As fruit from different districts and of differing maturities is known to have varying susceptibility to scald, it was of interest to compare the ability of oil wraps and DPA wraps to control scald in such varying lines of fruit. In both 1957 and 1958, samples of fruit were obtained from four sources, Auckland, two orchards in Hawke's Bay, and Nelson. Fruit was harvested at the same time in each district at weekly intervals during April. One-case samples from each harvest were wrapped in plain, oiled, or DPA (Australian) wraps respectively. All fruit was forwarded to Hastings for storage. Each pick from each orchard was examined independently.

TABLE 7. INCIDENCE OF SUPERFICIAL SCALD IN GRANNY SMITH APPLES WRAPPED IN THREE TYPES OF FRUIT WRAP

All results expressed as percentages
1 bushel per treatment

1957

Type of wrap	Harvest date	District							
		Auckland		Hastings No. 1		Hastings No. 2		Nelson	
		Exam'n. date	Scald	Exam'n. date	Scald	Exam'n. date	Scald	Exam'n. date	Scald
Plain Oiled Diphenylamine	8 Apr.	24 Aug.	98 91 1	27 Aug.	72 49 1	24 Aug.	96 87 0	27 Aug.	84 33 0
Plain Oiled Diphenylamine	15 Apr.	28 Aug.	100 72 0	10 Sep.	86 26 0	6 Sep.	100 70 9		
Plain Oiled Diphenylamine	22 Apr.	28 Aug.	92 67 1	21 Oct.	83 1 0	13 Sep.	93 29 3	16 Sep.	79 12 3
Plain Oiled Diphenylamine	29 Apr.	30 Aug.	82 94 0	21 Oct.	64 0 0	8 Oct.	94 24 0	27 Sep.	68 0 0
Number of apples per bushel			88 or 100		113		113		100 or 113

1958

Plain Oiled Diphenylamine	7 & 8 Apr.	4 Aug.	99 88 0	2 Sep.	70 15 0	1 Sep.	92 29 0	1 Sep.	97 4 0
Plain Oiled Diphenylamine	14 Apr.	12 Aug.	97 66 0	6 Oct.	83 35 0	8 Sep.	72 13 0	8 Sep.	88 16 0
Plain Oiled Diphenylamine	21 Apr.	15 Sep.	99 73 0	17 Nov.	92 24 0	14 Nov.	93 29 0	10 Oct.	86 0 0
Plain Oiled Diphenylamine	28 Apr.	22 Sep.	98 3 0	18 Nov.	86 5 0	14 Nov.	87 19 0	17 Nov.	97 15 0
Number of apples per bushel			88 or 100		100 or 113		113		100 or 113

Plain sulphite

Oil 13.9%

Diphenylamine 1 mg/100 sq. in.



Fig. 6.—Granny Smith apples. Lower fruit treated with DPA (500 p.p.m.)—no scald. Upper apples had been wrapped in oiled wraps—showing limited scald development.

Photo: J. Endt.

TABLE 8. APPLICATION OF 1,000 P.P.M. DIPHENYLAMINE, 5% ISOPROPYL ALCOHOL + 0.075% TWEEN 20 BY SPRAYING GRANNY SMITH APPLES LOOSE IN CASES, TO CONTROL SUPERFICIAL SCALD, 1958

Fruit from 4 orchards
10 loose bushels per treatment
Fruit dry into store

Orchard and Treatment	Harvest Date	Treat- ment Date	Examin- ation Date	Superficial Scald %		Diphenylamine Damage %
				Slight	Severe	
1 Check Sprayed	3 May	6 May	8 Oct.	26 0	64 0	22
2 Check Sprayed	12 May	13 May	16 Oct.	28 0	65 0	13
3 Check Sprayed	28 Apr.	29 Apr.	17 Oct.	36 0	38 0	3
4 Check Sprayed	30 Apr.	1 May	16 Oct.	25 0	61 0	20

Results for both years are presented in Table 7. In the Auckland-grown fruit in both years scald appeared earlier than in fruit from the other districts. The experiment clearly demonstrated the limitations of oiled wraps for scald control (Fig. 6), particularly in immature fruit. In some seasons oiled wraps fail to give adequate protection to the commercial Granny Smith crop, and in all probability this failure is connected with fruit maturity at harvest. In this experiment the outstanding result was the almost complete absence of scald in both seasons on all fruit of all maturities protected by DPA wrappers.

Results of spraying cases of loose-packed Granny Smith are given in Table 8. It is clear that DPA can be applied effectively by this method, and also that it is risky. The damage which occurred was extensive enough in some instances to make the affected fruit unmarketable. Damage occurred solely on the underside of the bottom layer, at contact points between fruit and timber, suggesting that drainage was incomplete.

TABLE 9. INCIDENCE OF SUPERFICIAL SCALD IN FRUIT DIPPED IN PHENOTHIAZINE
(Compare alternative treatments in Tables 2 and 3)

Variety	Year	Harvest Date	Treatment Date	Examination Date	Concentration p.p.m.	Superficial Scald %		Damage %
						Slight	Severe	
Delicious	1957	11 Apr.	13 Apr.	20 Nov.	1,000	5	0	
Dougherty	1957	6 May	8 May	6 Nov.	1,000	18	11	
Frimley Beauty	1957	24 Apr.	26 Apr.	2 Sep.	1,000	16	2	
Granny Smith	1957	29 Apr.	30 Apr.	1 Oct.	1,000	30	39	7
Granny Smith	1957	29 Apr.	30 Apr.	1 Oct.	2,000	25	61	74
Rome Beauty	1957	6 May	8 May	29 July	1,000	0	0	
Statesman	1957	24 Apr.	26 Apr.	19 Sep.	1,000	4	0	
Red Statesman	1957	14 Apr.	16 Apr.	23 Sep.	1,000	16	7	
Sturmer	1957	8 Apr.	9 Apr.	11 Nov.	1,000	22	7	
Ballarat	1958	28 Feb.	4 Mar.	4 Aug.	2,000	21	18	61
Cox's Orange	1958	17 Feb.	18 Feb.	5 May	2,000	25	30	
Granny Smith	1958	1 May	2 May	21 Oct.	1,000	50	39	
Granny Smith	1958	1 May	2 May	21 Oct.	1,000	57	13	
Granny Smith	1958				Un-treated	14	83	

Use of Phenothiazine

Smock (pers. comm.) suggested that phenothiazine with a similar chemical structure to DPA might also be able to control scald. Table 9 shows scald development in fruit which had been dipped in 1,000 and 2,000 p.p.m. phenothiazine. A comparison between these results and those presented in Tables 2 and 3 shows that phenothiazine did not

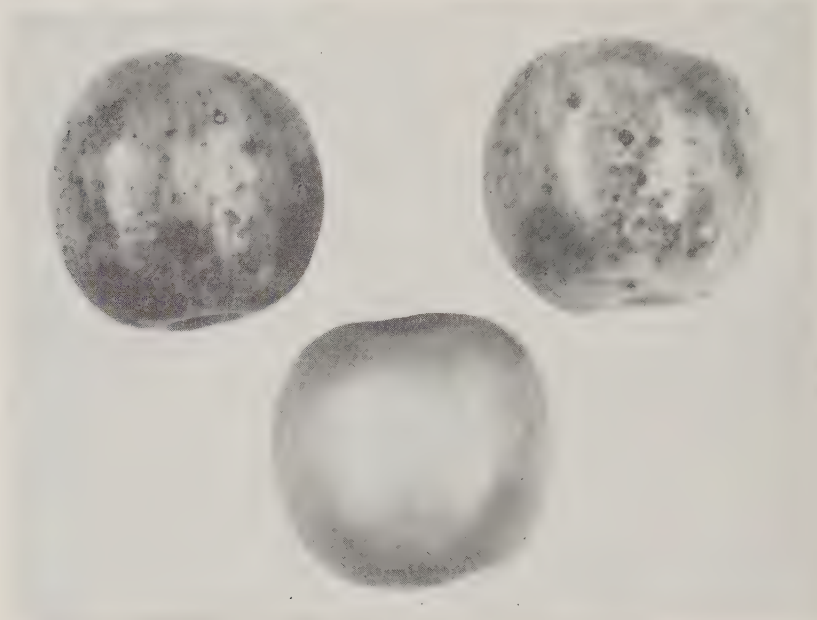


Fig. 7.—Granny Smith apples. Lower fruit treated with DPA (500 p.p.m.)—no scald. Upper fruit treated with phenothiazine (2,000 p.p.m.)—showing both scald and damage.

Photo: J. Endt.

compare favourably with DPA for scald control (Fig. 7), and that on some varieties damage occurred.

Effect of DPA on Other Storage Disorders

Because nothing was known of the effect of DPA on other storage disorders, detailed examinations for all disorders were made in the 1956 and 1957 dipping experiments (Table 10), and for the principle wrapping experiment conducted in 1957 and 1958 (Table 11). The only disorders that occurred in significant amounts were internal breakdown and core flush (brown core) (Padfield 1954).

Internal Breakdown. The results in Table 10 show little effect of DPA on incidence of internal breakdown. More numerous comparisons (Table 11) on the variety Granny Smith showed a tendency for fruit in DPA wraps to develop less breakdown than similar lots in plain or oiled wraps. Generally it appeared that breakdown is unlikely to increase significantly under DPA dipping treatments and when DPA was included in wraps breakdown in Granny Smith was apparently reduced, particularly under conditions of late harvest maturity and periods of longer storage duration, as in the third and fourth picks.

Core Flush. The results in Table 10 show that fruit treated with DPA in general developed less core flush than untreated fruit, particularly in the susceptible varieties Granny Smith and Statesman, although in the equally susceptible variety, Dougherty, reduction was

TABLE 10. INCIDENCE OF BREAKDOWN AND CORE FLUSH IN FRUIT TREATED WITH DIPHENYLAMINE

Variety	Breakdown %				Core Flush %			
	1956		1957		1956		1957	
	Un- treated	Dipheny- lamine	Un- treated	Dipheny- lamine	Un- treated	Dipheny- lamine	Un- treated	Dipheny- lamine
Ballarat	52	54	0	1	21	16	4	3
Cox's Orange			6	16			4	11
Delicious	0	0	16	14	1	0	2	1
Dougherty	0	0	1	0	78	69	63	56
Frimley Beauty	0	0	11	1	0	0	5	0
Granny Smith	36	7	10	4	88	35	65	44
Granny Smith				1				24
Granny Smith		39		1		40		27
Granny Smith	8	2			95	69		1st examination, wrap, 1.5 mg./100 sq. in. p.p.m.
Granny Smith		9				77		2nd examination, dip, 1,000 p.p.m.
Kidd's Orange								2nd examination, wrap, 1.5 mg./100 sq. in. p.p.m.
Red Statesman	29	22	9	3				1,000 p.p.m.
Red Statesman			16	8	68	32	2	2,000 p.p.m.
Rome Beauty	37	7	0	10	10	2	0	1st pick
Rome Beauty	1	1		0	0	0		2nd pick
Statesman	21	16	1	1	67	57	57	1,000 p.p.m.
Statesman				1				2,000 p.p.m.
Sturmer	15	4	7	18	26	6	13	2

TABLE 11. INCIDENCE OF INTERNAL BREAKDOWN AND CORE FLUSH IN GRANNY SMITH APPLES OF FOUR MATURITIES WRAPPED IN THREE TYPES OF FRUIT WRAP
1 bushel per treatment

Type of Wrap	Harvest Date	Internal Breakdown %										Core Flush %					
		District										District					
		Auckland		Hastings No. 1		Hastings No. 2		Nelson		Auckland		Hastings No. 1		Hastings No. 2		Nelson	
		1957	1958	1957	1958	1957	1958	1957	1958	1957	1958	1957	1958	1957	1958	1957	1958
Plain Oiled Diphenylamine	8 Apr. '57	13	9	0	0	0	2	37	8	43	44	71	54	74	58	91	98
	7 & 8 Apr. '58	27	12	0	0	3	0	17	2	83	35	77	45	89	58	83	83
		14	7	0	0	4	0	22	2	37	24	41	22	63	37	61	38
Plain Oiled Diphenylamine	15 Apr. '57	25	1	37	23	0	1	—	21	64	52	87	98	46	72	—	97
	14 Apr. '58	36	6	49	12	0	0	—	18	72	58	88	90	36	72	—	87
		12	2	14	7	0	1	—	1	34	36	60	64	1	33	—	87
Plain Oiled Diphenylamine	22 Apr. '57	7	40	11	66	4	76	14	37	50	99	77	100	48	100	30	100
	21 Apr. '58	23	34	12	55	0	62	3	41	80	99	62	99	24	99	19	100
		7	2	15	47	1	39	2	5	32	87	53	99	5	99	6	84
Plain Oiled Diphenylamine	29 Apr. '57	34	51	8	73	20	83	82	89	53	100	46	100	91	100	97	100
	28 Apr. '58	16	12	5	54	14	51	66	70	44	96	50	100	73	100	97	100
		19	3	4	36	8	22	49	64	39	94	22	97	72	52	96	100

Plain sulphite
Oil 13.9%
Diphenylamine 1 mg./100 sq. in.

not so marked. The results in Table 11 show a similar trend, but generally, as maturity at harvest increased and storage period lengthened, the effect of DPA decreased and in some instances disappeared altogether, as shown in picks three and four. Under no conditions was core flush increased by DPA.

Unsuccessful Treatments

The following materials were used as dips on the variety Granny Smith, but proved to be of little or no value for scald control.

Emulsions (3%) of mineral oils—Prorex M and S.1020, both oils fortified with antioxidants BHT (butylated hydroxytoluene) and BHA (butylated hydroxyanisole) either alone or in combination; Ionol C.P. (BHT) 1,000 p.p.m., and Santoquin (6-ethoxy-1,2-dihydro-2,2,4-trimethyl quinoline) 2,000 p.p.m.

DISCUSSION

Results of experiments described here clearly show the effective control of superficial scald which is possible using DPA, confirming results obtained with this chemical on other varieties in U.S.A. None of the other chemicals tested showed comparable properties. In Granny Smith, our most susceptible variety, more effective scald control can be obtained with DPA than by using oiled wraps. The mode of application of DPA to fruit does not appear to matter. The question of what is the best commercial method of application will depend on circumstances. Where fruit is packed, as for export, the use of DPA-impregnated wrapping papers will probably be the easiest method, but where fruit is handled loose and in bulk some form of spray application may prove desirable.

The residues of DPA on most of these experimental fruit samples have been determined and these have been reported separately (Harvey and Clark 1959). There is little doubt that, providing these residues prove acceptable to health authorities, the fruit industry should adopt the use of DPA instead of the currently used oiled wraps for the control of scald.

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A CLOVER MOSAIC VIRUS IN NEW ZEALAND PASTURES

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Summary

Clover mosaic virus is widespread in New Zealand infecting over 50% of plants in many pastures more than four years old. The virus has been found occurring naturally on white, red, subterranean, and strawberry clovers, broad bean, and sweet pea. It has been transferred experimentally to pea, bean, blue lupin, mung bean, cowpea but not to *Lotus uliginosus*, lucerne, cucumber, spinach, tobacco, or *N. glutinosa*.

The virus is readily transmitted experimentally by mechanical inoculation. Natural transmission occurs in the field in the absence of mechanical damage, suggesting the presence of an insect vector but the species involved has not yet been determined. Seed transmission did not occur.

From host range, symptoms, and physical properties (thermal inactivation 60° to 62°C, tolerance to dilution 10⁻⁶ but not 10⁻⁷), the virus appears to be closely related to pea-wilt virus of Johnson (1942).

No immunity has been found in 194 white-clover mother plants.

An antiserum has been prepared by injection of purified virus into a rabbit. The specific serological precipitate is the flocculent or H type suggesting the virus has a rod shaped particle.

In the glasshouse, infection with the virus caused a reduction in leaf yield of 24% in white, 55% in red, and 36% in subterranean clovers.

INTRODUCTION

The importance of clover plants in the grassland economy of New Zealand is well known and any reduction in clover growth could adversely affect pasture production. Virus diseases, known to reduce vigour of clover plants have been reported by Chamberlain (1936) who found pea mosaic on red clover, and by Fry (1952) who recorded lucerne mosaic virus on clover species at Palmerston North and on lucerne at Lincoln. The present work describes a third virus which is widespread in clovers in many districts. The name Clover Mosaic Virus is proposed for this virus which is closely related to the pea wilt virus which Johnson (1942) isolated from clovers in the U.S.

HOST RANGE AND SYMPTOMS

Natural Hosts

WHITE CLOVER (*Trifolium repens* L.). Symptoms on leaves of naturally infected plants appear as chlorotic markings following along veins or in interveinal areas (Fig. 1). Symptoms seldom occur on all leaves and are more noticeable on vigorously growing plants in the spring and autumn. Plants in recently grazed pastures usually show no leaf symptoms. Plants inoculated by leaf rubbing in the glasshouse develop chlorotic flecks along or between the veins of young leaves



Fig. 1.—Clover mosaic virus on white clover. Leaves from naturally infected plant.

seven to ten days after infection. This may persist, develop into a diffuse mottle, or disappear as leaves age. Infected plants are reduced in vigour.

RED CLOVER (*T. pratense* L.) The first symptom to appear is clearing of the veins of young leaves. Later, pale green or yellowish areas spread out from the veins to form a mosaic of light and darker green areas (Fig. 2). This pattern occurs regularly on infected plants and is not seasonal or affected by grazing as in white clover. In the glasshouse a marked reduction in leaf size and plant vigour occurs.

SUBTERRANEAN CLOVER (*T. subterraneum* L.) Variety Mt. Barker: Vein clearing which appears five to seven days after inoculation is soon replaced by chlorotic mottling of leaves which are reduced in size and may become puckered. Plants become stunted.

Variety Talarook: Soon after the onset of vein clearing, the veins of young leaves become necrotic. Necrosis spreads throughout the plant resulting in death.

STRAWBERRY CLOVER (*T. fragiferum* L.). Vein clearing appears on newly developed leaves, fades as leaves age, and usually disappears completely leaving the old leaves symptomless. Plants are reduced in vigour.

BROAD BEAN (*Vicia faba* L.). Naturally infected plants develop pale green or yellowish spots or ringspots on leaves. Plants are reduced in vigour. When inoculated by leaf rubbing in the glasshouse, necrotic spots or ringspots appear on the inoculated leaf surface after



Fig. 2.—Clover mosaic virus on red clover. Leaves showing systemic mottle 14 days after inoculation.

five to seven days (Fig. 3). Seven to 14 days later pale green or yellowish spots or ringspots develop on young leaves.

SWEET PEA (*Lathyrus odoratus* L.). Naturally infected plants collected in Auckland showed no leaf symptoms. Pale-coloured or whitish streaks occurred on the flower petals which were also crinkled.



Fig. 3.—Clover mosaic virus: Local lesions on leaf of broad bean,

Although clover mosaic virus was recovered from these plants, there is no evidence it was the only virus present. It may not, therefore, have been the sole cause of the flower symptom.

Experimental Hosts

PEA (*Pisum sativum* L.). Similar symptoms are produced on varieties Greenfeast, Onward, William Massey, Greencrop, William Hurst, and Little Marvel. Plants inoculated when three leaves are well expanded develop brown, sunken, necrotic local lesions after four to seven days. About the same time vein clearing appears on the next two leaves, the lower of the two being curled downwards (Fig. 4). These five leaves later all die and remain hanging from the ends of the petioles. Later-developed leaves may show a mild blotch-type mottle (Fig. 4A) or remain symptomless. Plants become stunted in growth. Sometimes stem necrosis follows the development of local lesions and the plants die.



Fig. 4.—Clover mosaic virus: Necrosis of inoculated leaves of Greenfeast pea 11 days after inoculation.

BEAN (*Phaseolus vulgaris* L.). All varieties tested were susceptible. They were Black Valentine, Canadian Wonder, Masterpiece, Refugee Stringless, Stringless Greenpod, Surecrop Wax, Surprise, Sydney Wonder, Tendergreen. Ten to 14 days after inoculation veins of young leaves become yellowed and translucent (Fig. 5). The yellowing is often confined to a few small areas of fine veins but may involve the whole vein network over most of the leaf. Infected plants are slightly reduced in vigour.



Fig. 4A.—Clover mosaic virus: Mottling on leaves of Onward pea 7 weeks after inoculation.



Fig. 5.—Clover mosaic virus: Clearing of veins of bean.

BLUE LUPIN (*Lupinus angustifolius* L.). Ten to 14 days after inoculation mild pale-green mottling appears on young leaves. A few days later the inoculated leaves become necrotic and die. Later-

developing young leaves are narrowed and mottled, and develop necrotic spotting.

MUNG BEAN (*Phaseolus aureus* Roxb.). Four or five days after inoculation reddish brown necrotic local lesions appear on inoculated leaves (Fig. 6). The lesions do not enlarge nor does the virus invade the plant systemically.



Fig. 6.—Clover mosaic virus: Necrotic lesions on leaf of mung bean, healthy leaf on right.

COWPEA (*Vigna sinensis* (L.) Savi.). Ten to 14 days after inoculation, vein clearing, similar in distribution to that on beans, occurs on some young leaves (Fig. 7). A more severe vein clearing involving the whole vein network usually develops on leaves of lateral shoots which are small and may be twisted.

INSUSCEPTIBLE SPECIES

Attempts to transmit the virus to the following species by mechanical inoculation were unsuccessful: *Lotus uliginosus* Schkuhr., *Medicago sativa* L., *Cucumis sativus* L., *Nicotiana tabacum* L., *N. glutinosa* L., *Spinacea oleracea* L.



Fig. 7.—Clover mosaic virus: Clearing of veins of cowpea.

METHODS OF TRANSMISSION

Sap Inoculation. The virus is readily transmitted by leaf rubbing, using an abrasive.

Insects. White- and red-clover plants raised from seed in an insect-free glasshouse were set out in a field of white clover containing a high percentage of infected plants. The test plants were prevented from touching field plants by maintaining a bare area of ground for two feet on either side of the row. No grazing animals entered the field nor were the plants handled during the experiments. When the field was mown the row of test plants was avoided. After six months, symptoms had appeared on three out of 20 red clovers, and serological test showed that 10 out of 53 white clovers were infected. This result strongly suggests the presence of an insect vector in the field.

Preliminary attempts to transmit the virus with the aphids *Myzus persicae* Sulz., *M. ornatus* Laing., and *Aulacorthum solani* (Kltb.) using feeding times of five to ten minutes or several days have failed.

Seed Transmission: Of 812 white-clover plants raised from seed saved from three infected plants, none developed infection. It is therefore unlikely that the virus is seed transmitted, at least in white clover.

PROPERTIES OF THE VIRUS

The virus in sap from infected pea plants was still infectious after heating for 10 minutes at 60°C but not at 62°C, and was infectious at a dilution of one in a million but not at one in ten million. Studies on the purification and properties of the virus have been made by Fry, Grogan, and Lyttelton (in press).

PREPARATION OF ANTISERUM

Two ml of a virus preparation was injected intraveinously into a rabbit at weekly intervals for four weeks. The rabbit was bled 10 days after the last injection. The specific precipitate formed when virus and antiserum were mixed was of the H type similar to that produced by rod-shaped tobacco mosaic virus and its antiserum. This suggests that the clover mosaic virus particle, like TMV, is rod shaped.

Use of the antiserum has proved a convenient rapid method for diagnosis of specimens collected in the field. For this purpose the slide agglutination technique is used. No agglutination occurs with infective clover or pea sap unless it has been held for about two hours at room temperature or heated to 40°C.

IDENTITY

The virus described above has a host range similar to pea wilt virus of Johnson (1942) and produces similar symptoms in most hosts. Differences occur in mung bean where the New Zealand virus does not cause systemic infection and in cowpea where local lesions do not precede systemic infection. Neither virus is transmissible to *Medicago sativa*, *Cucumis sativus*, *Nicotiana tabacum*, *N. glutinosa*, *Spinacea oleracea*. The reported differences in dilution end point (pea wilt 10^{-5} New Zealand virus 10^{-6}) and thermal inactivation (pea wilt 58°–60°C, New Zealand virus 60°–62°C) do not preclude a close relationship.

Thus the clover mosaic virus described here appears to be closely related to pea wilt virus (Johnson 1942). To avoid confusion with the fusarium wilt of pea (*Fusarium orthoceras* Appel. et Wollenw. var *pisi* Inf.) known locally as pea wilt, it is suggested that the common name Clover mosaic virus be adopted.

INCIDENCE

Clover mosaic virus has been found in all districts in which clovers have been examined; Northland, Auckland, South Auckland, Waikato, Bay of Plenty, Hawke's Bay, Manawatu, Taranaki, Nelson, Marlborough, and mid Canterbury. Infection is usually absent from or rare in recently sown pastures but is often present in over 50% of plants in pastures more than four years old. However, pastures may develop a high incidence of infection relatively rapidly. A red-clover stand in a corner field near Palmerston North was examined eighteen months after sowing. Infection was present in all samples examined up

to 100 yd from each road boundary. Volunteer white clovers growing on the adjacent roadsides were also infected. These may have been a source of infection. Roadside clover plants are commonly infected in all the above districts. White clover is more often infected than other clover species, probably because of its more truly perennial growth.

In species other than clover, natural infection has been observed in broad bean and sweet pea plantings in Auckland.

SUSCEPTIBILITY OF WHITE-CLOVER SELECTIONS

Of 194 white-clover mother plants maintained by Grasslands Division at Palmerston North, 180 gave a positive serological test for clover mosaic virus. The remaining 14 were tested by sap inoculation and a further eight found to be infected. Cuttings were grown from the remaining six plants and inoculated with the virus. Subsequent inoculation to bean and pea showed that all plants had become infected. This shows that there is no source of immunity to clover mosaic virus in the above breeding material.

Most of the mother seed-plants were probably already virus infected when they were selected for high yield. This has very likely resulted in the development of strains of white clover relatively tolerant to the disease. This may account for the mild symptoms shown by pedigree lines of white clover in the field and the ease with which symptoms become masked.

EFFECT ON YIELD AND NITROGEN CONTENT

The effect of clover mosaic virus on leaf yield was studied in the glasshouse since natural spread of infection would have made trials in the field impractical.

White, red, and subterranean clover plants were raised from certified seed lines and transferred into four-inch pots which were held in trays on the glasshouse bench. When established, half of the plants were inoculated with clover mosaic virus and then rearranged in the trays, so that three infected were adjacent to three healthy. Eight repeats of each such unit were obtained for each clover species. Top growth was harvested by cutting one inch above soil level. Four cuts were made at intervals of five weeks commencing one month after inoculation.

Results summarised in Table 1 show that there was a reduction in yield amounting to 24% for white, 36% for subterranean, and 55% for red clover.

Determination of total nitrogen by a Kjeldahl procedure was carried out on the first two cuts. Results in Table 1 show that for white and subterranean clover there was little difference in nitrogen content as percentage of dry weight in healthy and infected plants. In red clover there was a gain in nitrogen as percent of dry weight in infected plants. In all three species, however, the total nitrogen per plant was considerably decreased in the infected plants.

TABLE 1. LEAF WEIGHTS AND NITROGEN CONTENT FROM HEALTHY AND VIRUS-INFECTED CLOVERS

Type of Clover	Cut No.	Healthy				Infected				Healthy minus Infected			
		Fresh Weight g	Dry Weight g	N % of DW	N Total g	Fresh Weight g	Dry Weight g	N % of DW	N Total g	% FW	% DW	% Total N	% Total N
White	1	34.9	3.70	2.7	.101	28.1	3.02	3.04	.092	19.5**	18.5**	8	8
	2	46.5	4.62	3.6	.165	41.1	4.00	3.56	.142	11.6	13.4*	13	13
	3	58.7	5.69			51.6	4.35			24.9	23.6*		
	4	45.5	5.24			26.8	3.18			41.1*	39.4*		
	Mean	48.1	4.75			36.4	3.63			24.3**	24.2**		
Red	1	39.6	4.74	2.69	.127	18.8	2.34	3.73	.098	52.5**	50.8**	23	23
	2	35.5	5.79	2.88	.119	17.9	3.78	3.83	.081	49.6**	34.7**	31	31
	3	46.1	4.79			17.4	1.83			62.3**	62.0**		
	4	19.7	2.25			9.6	1.08			51.3**	52.0**		
	Mean	35.5	3.98			16.1	1.84			54.6**	48.8**		
Subterranean	1	35.5	4.31	3.36	.145	31.0	4.27	3.29	.140	15.2*	1.0	3	3
	2	34.5	6.08	2.86	.171	45.6	5.38	2.84	.153	16.0**	11.5*	13	13
	3	62.7	7.85			27.6	3.78			56.0**	39.2**		
	4	46.7	5.72			20.6	2.76			55.9**	51.8**		
	Mean	49.0	5.99			31.2	4.05			36.3**	32.5**		

* Significant at 5% level.

** Significant at 1% level.

DISCUSSION

The widespread incidence of the virus suggests that it has been well established in clovers for many years. The effect it has had or will have on the yield of pasture plants is difficult to assess. Reduction in yield of leaf in the glasshouse has been demonstrated. If this occurs to the same extent in the field a reduction of 25% in yield of white and 50% of red clover could be expected in pure stands which were 100% infected. In trials conducted by Sears (1953), the yield from white clover in a grazed pasture of ryegrass and white clover with return of dung and urine was 40% of the total. Allowing for 100% infection and 25% yield reduction in white clover, this would give a 10% overall reduction in yield from the pasture.

The effect of reduction in vigour of the clover on its ability to maintain itself in competition with grass species of the pasture and with healthy clovers is not known. If the infected clovers were unsuccessful in the competition for space their loss would be offset on a yield basis by the grasses or new seedling clovers that took their place. The loss or reduction in numbers of clovers would result in a loss of nitrogen fixation.

Thus detailed studies on the ecology of grasslands containing virus-infected clover plants would be required before any valid assessment of the importance of the disease in our grassland economy could be made.

ACKNOWLEDGEMENTS

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A BROAD-SPECTRUM ANTIBIOTIC PRODUCED BY A WHITE-SPORED STREPTOMYCETE (ALBUS GROUP) ISOLATED FROM NEW ZEALAND SOIL

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(Received for publication, 6 July 1959)

Summary

The morphological, cultural, and physiological characteristics of a white-spored streptomycete isolated from New Zealand soil are described.

Fermentation procedures are given for the production of an antibiotic active against both gram-positive and gram-negative bacteria.

The antibacterial spectra of crude culture filtrates and of the partly purified antibiotic are given.

INTRODUCTION

In the course of a general study of the streptomycetes in New Zealand soils, it was a matter of routine to test isolates for antagonistic properties.

From a sample of Tokomaru silt loam collected near Palmerston North, one rather small and slow-growing colony produced a relatively large zone of inhibition against both gram-positive and gram-negative test organisms. The streptomycete was picked out for further study and the results are set out below.

DESCRIPTION OF ORGANISM

Morphological

- (a) Vegetative mycelium hyaline, branched, about $0.4\ \mu$ in diameter.
- (b) Aerial mycelium abundant, normally white, but with faint brownish tint on some media. Branches of sporophores end in sinistrorse, loose, open spirals (Fig. 1). Number of turns variable, but usually 4 to 6. Diameter of spirals $5\ \mu$ approximately. Spores variable in size, but normally oval about $0.8\ \mu \times 0.5\ \mu$ (Fig. 2).

Cultural and Physiological

Synthetic agar (Czapek) (Waksman 1950). Colonies small, diameter about 2 mm. Aerial mycelium white, well developed, with loose cottony texture. Margin irregular with submerged hyphae radiating beyond aerial mycelium. Reverse colourless or off white. Incubation 14 days at 27°C . See Fig. 3a.

Starch agar B (Waksman 1950). Colonies about 4 mm in diameter. Slightly raised at centre. Surface matted and pitted from exudation drops. Exudate colourless. Aerial mycelium cottony white, turning light cream towards centre of colony. Submerged hyphae extending



Fig. 1.—Edge of colony on Czapek agar showing loose, open spirals.

beyond aerial mycelium. Reverse white turning light brown at centre. Incubation 14 days at 27°C. See Fig. 3b.

Glucose peptone agar B (Waksman 1950). Colonies raised 3.5–4.5 mm in diameter. Aerial mycelium plentiful, cottony white. Exudation drops present. Exudate colourless. Submerged hyphae not extending beyond aerial mycelium. Reverse off white to light cream. Incubation 14 days at 27°C. See Fig 3c.

Emerson's agar (Gottlieb et al. 1948). Colonies about 7–8 mm in diameter, raised, cracked, and folded. Margin irregularly lobed. Aerial hyphae white, short, and matted. Exudation drops present. Exudate colourless. Submerged hyphae not extending beyond aerial mycelium. Reverse brown at centre, paling towards edge of colony. Incubation 14 days at 27°C. See Fig. 3d.

Potato plug. Growth good. Aerial mycelium white. No soluble pigment.

Carrot plug. Growth good. Aerial mycelium white. No soluble pigment.

Calcium malate agar (Krainsky 1914). Aerial mycelium white to light grey. Reverse yellow gold. No soluble pigment. Medium cleared about 4 mm beyond edge of colony.

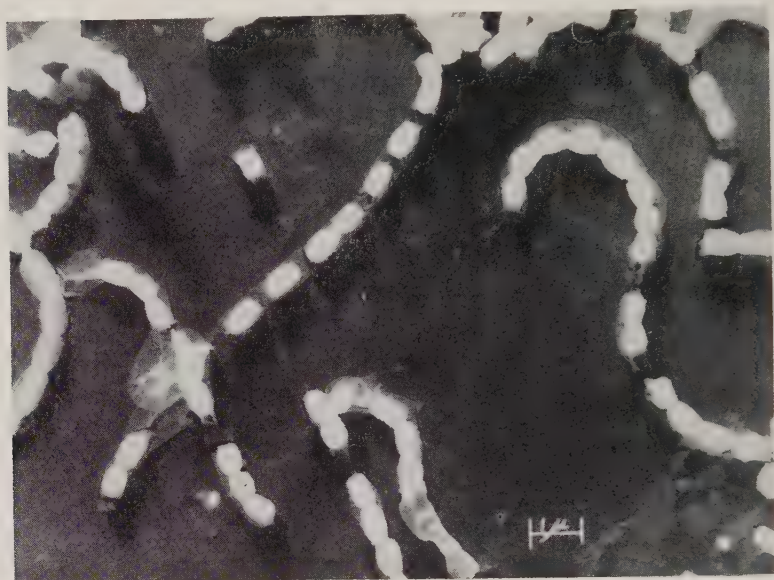


Fig. 2.—Electron micrograph showing spores. Preparation made from starch agar culture after 14 days at 27°C.

Glucose broth (Waksman 1950). Growth good. Pellicle formed. No soluble pigment.

Litmus milk. Light pellicle or ring formed. Peptonisation with alkaline reaction.

Gelatin. Hydrolysis rapid. Growth cream coloured. No soluble pigment.

Carbohydrate utilisation (Pridham and Gottlieb 1948). Good growth on xylose, rhamnose, cellobiose, arabinose, galactose, mannitol. Slight growth on raffinose, inositol, inulin, salicin. No growth on lactose, sorbitol, dulcitol.

Starch. Hydrolysed.

Casein. Hydrolysed.

Nitrate. Reduction to nitrite and ammonia.

Optimum temperature. Circa 27°C. No growth at 37°C.

Antagonistic properties. Active against both gram-positive and gram-negative bacteria.

FERMENTATIONS

Fermentations were carried out in shake flasks, using one-litre flasks containing 200 ml of medium, or 500-ml flasks with 100 ml of medium. The flasks were placed on an oscillating shaker with a horizontal throw of 2½ inches and working at the rate of 90 oscillations per minute. The temperature was controlled at 27°C. Fermentation

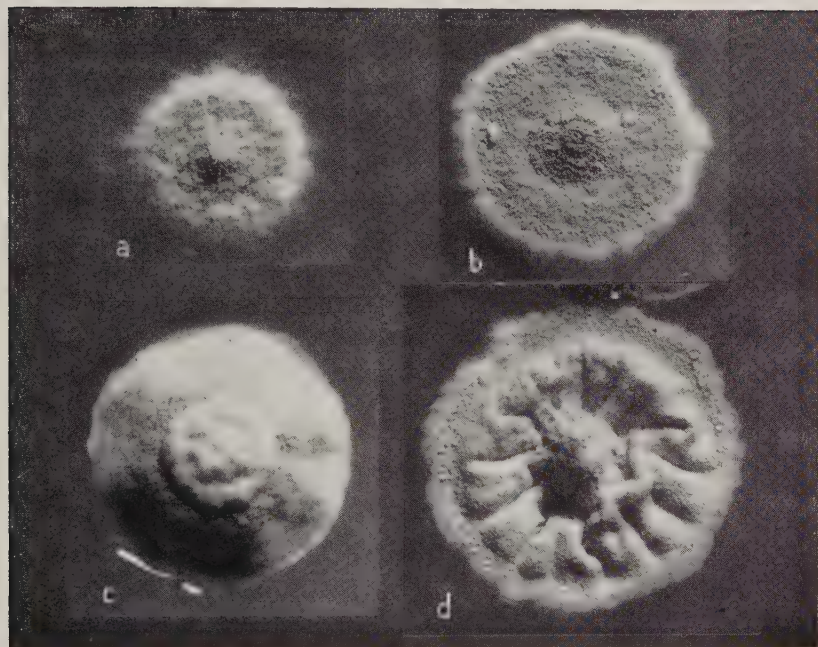


Fig. 3.—Variation in colony characteristics on different media after 14 days at 27°C. (a) Synthetic agar (Czapek). (b) Starch agar. (c) Glucose peptone agar. (d) Emerson's agar.

times varied with the size of inoculum and the constitution of the medium. Normally the highest antibiotic titres were reached between 90 and 120 hours.

Medium

After a number of preliminary trials to determine a suitable fermentation medium, the following was selected:

Soya-bean meal (defatted)	10.0 g
Dextrose	10.0 g
Sodium chloride	5.0 g
Calcium carbonate	1.0 g
Water	1 litre

The medium was adjusted to give a pH of 6.5 after sterilisation. Sterilisation was minimal, 10 minutes at 12 lb pressure, as prolonged heating at pressure resulted in reduced antibiotic titres.

Inoculum

The inoculum consisted of a spore suspension prepared by transferring surface growth from agar cultures to sterile water and shaking with glass beads. The resulting suspension was adjusted against standard opacity tubes to give a spore count of about 50,000,000 spores per ml. This was later checked by dilution plates to determine the viable spore count. One millilitre of spore suspension was normally used for each 500-ml flask.

Course of Fermentation

In a typical fermentation there is a steady rise in pH during the first 72–96 hours, reaching a peak of *ca.* pH 8. During this phase, growth is rapid and the organism forms tiny pellets. The medium begins to clear and develop a faint straw colour.

During the next 24 hours the pH falls sharply and antibiotic production reaches its maximum. The medium is now clear and straw coloured, and the organism completely pelleted. At this stage the fermentation is harvested.

If the fermentation is continued there is a rise in pH and the antibiotic titre begins to fall. On occasion quite good titres were obtained from broths harvested after the pH had risen, but delay at this point normally resulted in reduced yields (Fig. 4).

Amount of Inoculum

The effect of varying the amount of inoculum is shown on Fig. 5. Within limits, the duration of the fermentation could be reduced by increasing the amount of inoculum. The level of antibiotic production remains fairly constant, regardless of the amount of inoculum used.

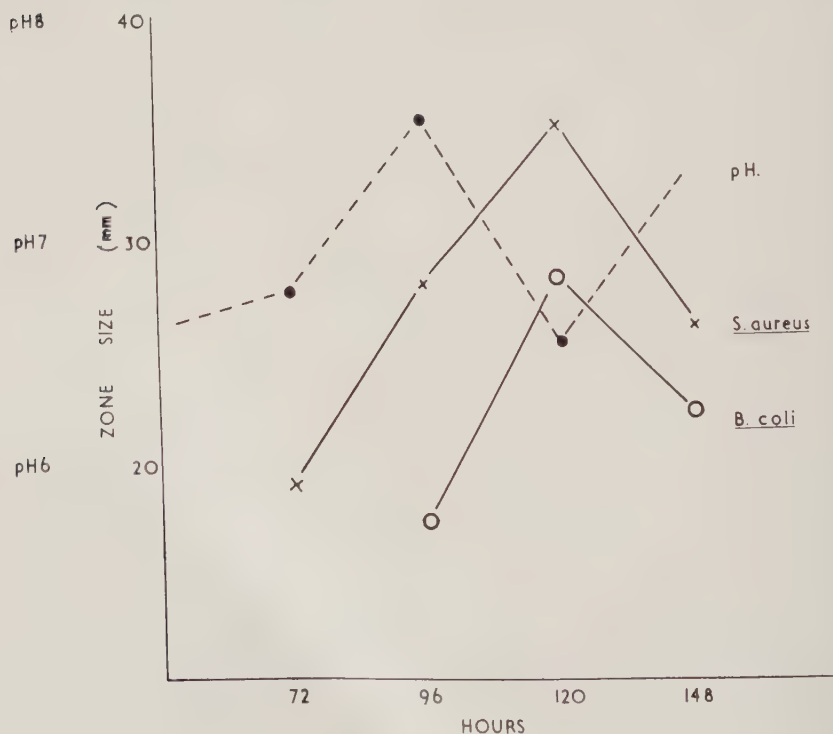


Fig. 4.—Changes in pH in relation to antibiotic production.

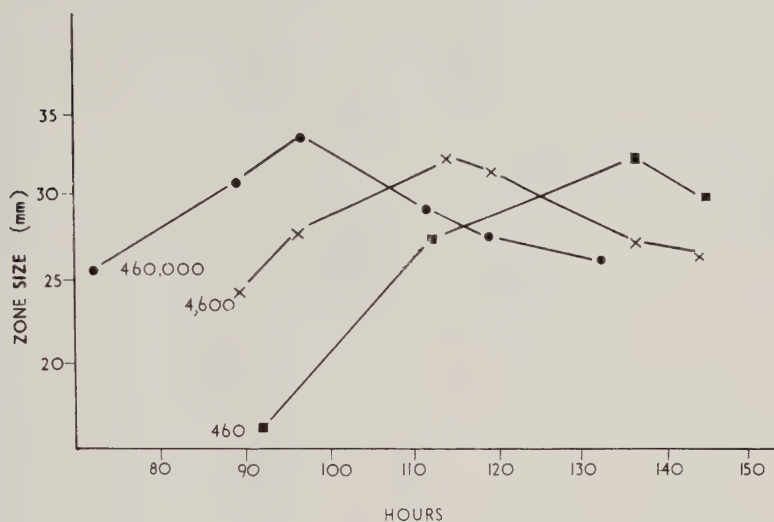


Fig. 5.—The effect of size of inoculum (spores/ml).

ANTIBIOTIC SPECTRUM

The antagonistic properties of the culture filtrates were determined against a number of test organisms. The organisms for test were streaked on the surface of nutrient agar to which had been added varying amounts of culture filtrate, previously adjusted to neutrality, to give a series of dilutions up to one part in three thousand, 1 : 3000. Other methods of assay were also used. Results were recorded after 48 hours' incubation at temperatures appropriate to the organism tested.

Stability of Antibiotic

Culture filtrates adjusted to pH 3 lost little activity when heated to 98°C for 15 minutes, but activity decreased as the pH was raised. Stored at 11°C a filtrate at pH 2.8 showed no loss of potency after 5 months' storage.

Concentration of Antibiotic

Deep fermentations of 100–130 litres provided material for this work. The following method gave a concentrated product containing about 60% of the original activity.

Preliminary concentration was affected by freezing. The broth is then adjusted to pH 7.5, heated to 40°C, and stirred for 5 minutes with lissapol. The amount of lissapol used is 1% by volume. The mixture is then cooled, mixed with 20–30% by volume of chloroform, and passed through a separator to remove the chloroform portion. This extract contains a complex of antibiotic with the sulphonated fatty compounds of the lissapol. The chloroform extract is quickly brought to pH 10 to decompose the complex and the antibiotic hydrochloride formed immediately by adding HCl to pH 2. By forming an emulsion with water (10% of original broth volume) the antibiotic is passed to

TABLE 1. INHIBITORY EFFECT AT VARIOUS DILUTIONS AGAINST A VARIETY OF TEST ORGANISMS

Test Organism	Concentration of Culture Filtrate in Medium											
	1 ₁₀	1 ₂₀	1 ₄₀	1 ₈₀	1 ₁₀₀	1 ₂₀₀	1 ₄₀₀	1 ₆₀₀	1 ₈₀₀	1 ₁₀₀₀	1 ₂₀₀₀	1 ₃₀₀₀
<i>S. aureus</i>	—	—	—	—	—	—	—	—	—	—	—	—
<i>S. typhimurium</i>	—	—	—	—	—	—	—	—	—	—	—	—
<i>B. coli</i>	—	—	—	—	—	—	—	—	—	—	—	—
<i>B. subtilis</i>	—	—	—	—	—	—	—	—	—	—	—	—
<i>B. pneumoniae</i>	—	—	—	—	—	—	—	—	—	—	—	—
<i>B. proteus</i>	—	—	—	—	—	—	—	—	—	—	—	—
<i>M. phlei</i>	—	—	—	—	—	—	—	—	—	—	—	—
<i>B. mesentericus</i>	—	—	—	—	—	—	—	—	—	—	—	—
<i>B. cholerae</i>	—	—	—	—	—	—	—	—	—	—	—	—
<i>B. megatherium</i>	—	—	—	—	—	—	—	—	—	—	—	—
<i>B. mycoides</i>	—	—	—	—	—	—	—	—	—	—	—	—
<i>S. lutea</i>	—	—	—	—	—	—	—	—	—	—	—	—
<i>S. citrea</i>	—	—	—	—	—	—	—	—	—	—	—	—
<i>B. aerogenes</i>	—	—	—	—	—	—	—	—	—	—	—	—
<i>A. tumefaciens</i>	—	—	—	—	—	—	—	—	—	—	—	—
<i>E. amylovora</i>	—	—	—	—	—	—	—	—	—	—	—	—
<i>E. carotovora</i>	—	—	—	—	—	—	—	—	—	—	—	—
<i>Ps. pisi</i>	—	—	—	—	—	—	—	—	—	—	—	—
<i>X. michiganense</i>	—	—	—	—	—	—	—	—	—	—	—	—
<i>Ps. medicaginis</i>	—	—	—	—	—	—	—	—	—	—	—	—

Key — no growth
 + slight growth
 ++ fair growth
 +++ good growth
 ++++ growth equal to control

the aqueous phase and separated as before. This procedure is repeated. The extract is evaporated *in vacuo* to dryness and extracted with absolute methanol. The methanol extract is taken to dryness and the residue taken up in a minimum quantity of water and freeze dried.

Antagonistic Properties of Concentrated Antibiotic

The concentrated antibiotic was assayed by streaking the test organisms on the surface of nutrient agar in which the antibiotic was incorporated at various concentrations. Table 2 gives the minimum inhibitory concentrations in $\mu\text{g/ml}$.

TABLE 2. MINIMUM INHIBITORY CONCENTRATIONS IN $\mu\text{g/ml}$ OF CONCENTRATED ANTIBIOTIC

Test Organism	Inhibitory Concentration $\mu\text{g/ml}$	Test Organism	Inhibitory Concentration $\mu\text{g/ml}$
<i>B. coli</i>	2.5 -6.6	<i>M. phlei</i>	
<i>B. subtilis</i>		ATCC 355	2.5 -6.6
ATCC 6633	0.5 -2.5	<i>B. megatherium</i>	0.5 - 1.0
<i>K. pneumoniae</i>		<i>B. mycoides</i>	< 0.05
ATCC 9997	1.0 -2.5	<i>S. lutea</i>	< 0.05
<i>B. proteus</i>	12.5 -2.5	<i>S. citrea</i>	0.5 - 0.125
<i>S. aureus</i>	0.125-0.25	<i>A. tumefaciens</i>	2.5 - 6.6
<i>B. aerogenes</i>	2.5 -6.6	<i>E. amylovora</i>	1.0 - 2.5
<i>S. typhimurium</i>	2.5 -6.6	<i>E. caratovora</i>	12.5 -25.0
<i>S. choleraesuis</i>	1.0 -2.5	<i>Ps. pisi</i>	2.5 - 6.6
<i>B. mesentericus</i>	2.5 -6.6	<i>X. michiganense</i>	1.0 - 2.5
		<i>Ps. medicaginis</i>	0.25- 0.5

Toxicity

Preliminary trials with mice and rabbits indicated high toxicity resulting in extensive damage to liver and kidneys.

ACKNOWLEDGEMENTS

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BLOAT IN CATTLE

XVI. BOVINE SALIVA: THE CHEMICAL COMPOSITION OF THE PAROTID, SUBMAXILLARY, AND RESIDUAL SECRETIONS

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Summary

The saliva from the parotid and submaxillary glands and the residual saliva that flowed from the mouth, have been collected from 5- to 8-months-old calves and analysed for electrolytes and nitrogenous compounds. Carbachol and inflation of the rumen have been used to induce salivation.

Parotid and residual saliva contained Na^+ and HCO_3^- at concentrations of about 100 me./litre, potassium and phosphate at approximately 15–20 me./litre, while calcium was in the range 3–7 me./litre. The nitrogen of parotid saliva was mainly in the form of urea. Residual saliva contained in addition appreciable concentrations of mucoprotein. The compositions of both secretions were independent of the type of stimulation used.

The mucoprotein content of submaxillary saliva was greater than that of either parotid or residual saliva, but the urea concentration did not differ significantly.

Submaxillary saliva induced by carbachol stimulation contained less Na^+ and HCO_3^- and more Ca^{2+} than the parotid and residual saliva. The content of potassium was approximately the same but HPO_4^{2-} was present only in traces.

Inflation of the rumen produced approximately a fivefold increase in the mucoprotein concentration of submaxillary saliva without significantly altering the concentration of other components.

The possible relationship between the composition of saliva and production of bloat are discussed.

INTRODUCTION

Ruminant saliva is complex in origin and composition. Besides the secretions from the parotid, submaxillary, and sublingual glands, there is a considerable amount of fluid from glandular tissue in the mouth and this "residual" saliva (other than that from the parotid and submaxillary glands) is rather complex in origin (Kay, pers. comm.). It is possible, and has been done in one experiment reported here, to eliminate sublingual saliva from the residual saliva. As these glands secrete very little during experiments of this nature, it makes little

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difference to the results whether their secretions are or are not included in the residual saliva. Early workers, particularly Colin (1886), and Scheunert and Trautman (1921), showed that the secretion from the parotid glands was continuous and the rate increased when the animal was feeding. This has been amply confirmed by recent studies (McDougall 1948; Coats *et al.* 1956; Denton 1958). Colin (1886) claimed that the sublingual glands also secreted continuously, while Scheunert and Trautman (1921) found that with sheep the submaxillary glands secreted only while the animal was eating.

Ruminant saliva performs functions other than wetting the food and acting as a lubricant for the food bolus, as Scheunert and Trautman (1921) found that the loss of the secretion from one parotid gland was sufficient to cause nutritional failure and death of the sheep. Denton (1956) found that the effects of loss of parotid saliva could be attributed to sodium depletion. Potassium largely replaced sodium in the parotid saliva and the quantity of sodium excreted in the urine fell to negligible amounts. The provision of an adequate supply of sodium bicarbonate reversed these changes.

In contrast to monogastric species, the saliva of ruminants is quite alkaline. Markoff (1913) calculated that in cattle the total secreted per day was equivalent to 300–350 g of sodium bicarbonate. McDougall (1948) found that the saliva of sheep could be described as a bicarbonate-phosphate buffer. Turner and Hodgetts (1955) concluded that the volatile fatty acids produced in the rumen would lower the pH to a value below 3.0, if no saliva was secreted.

The reticulo-rumen has no glandular tissue as such and the large volume of saliva secreted is important in maintaining the well buffered fluid conditions necessary as a suitable environment for the rumen micro-organisms. The volume of saliva secreted daily by cattle has been estimated by Colin (1886) at 56 kg and by Markoff (1913) at 50 litres. Balch (1954) has shown that for each 10 lb of food eaten the cow secretes 45–55 lb saliva with hay, 12–15 lb with ground concentrates, and 7.5–10 lb with grass. McDougall (1948) and Scheunert and Trautman (1921) found considerable concentrations of nitrogen in ruminant saliva, largely as urea, a form readily utilised by rumen micro-organisms and, as pointed out by McDonald (1948), this may be important in the survival of ruminants if the nitrogen content of the food is very low.

There is little information on the chemical composition of the individual salivary secretions. The only comprehensive work is that of McDougall (1948) which is confined to the analysis of parotid and mixed saliva of sheep.

The present work was carried out as part of an investigation on bloat in cattle. Work in this laboratory has strongly indicated that this disorder is caused by foaming of the rumen ingesta. Fermentation gases are retained in the rumen in the form of a stable foam which cannot be eliminated by eructation (see Johns 1956, 1958). It has been shown (Mangan 1959) that the mucoprotein of saliva aspirated from under the tongue of cattle is a powerful foaming agent and so may

have some significance in bloat. Reid (unpublished) has shown that bloat in cattle occurs only when the pH of the rumen liquor is below about 6.3. The parotid saliva of sheep has been found by McDougall (1948) to be high in bicarbonate and phosphate at about pH 8. Saliva would thus tend to prevent bloat by buffering a drop in pH. On the other hand, if the pH in the rumen should drop to about pH 6, the bicarbonate of the saliva would decompose, evolving carbon dioxide gas, and if bloating conditions prevailed, this would aggravate the position. One volume of parotid saliva containing 0.1M bicarbonate under these conditions would evolve approximately 2 volumes of gas on entering the rumen.

Inflation of the rumen to pressures of 5–15 mm Hg causes an increased salivary flow in some calves (Phillipson and Reid 1958); further distension inhibits this response. This effect appears to depend on whether or not gas or food enters the thoracic part of the oesophagus, as distension of the rumen alone, if the oesophagus is tied at the cardia, is inhibitory to salivation but distension of the thoracic oesophagus stimulates profuse salivation (Kay and Phillipson 1959). The possibility exists that excessive salivation may occur during the early stages of bloat if foaming material enters the oesophagus, and this paper is concerned with the composition of saliva secreted after inflation of the rumen or stimulation in other ways.

METHODS

Jersey calves, 5–8 months old, were anaesthetised with approximately 1% chloralose in saline (50 mg/kg body weight). The trachea and rumen were cannulated and the cervical oesophagus ligated. In some experiments the parotid and submaxillary ducts on one side only were cannulated, but in others the pairs of ducts were cannulated and the saliva from each pair was combined. In the latter case the saliva flowing from the mouth was termed "residual". In one experiment the ducts to the sublingual glands were also cannulated, but the secretion was too small to affect the composition of the residual saliva. Nitrogen or carbon dioxide was used for inflation of the rumen.

Total nitrogen was estimated by the micro-Kjeldahl method of Chibnall *et al.* (1943); *urea* by incubation with urease and estimation of ammonia by the Conway (1939) micro-diffusion method; *protein nitrogen* either by exhaustive dialysis followed by total-nitrogen determination, or, when adequate quantities were available, by precipitation with 32% w/v anhydrous sodium sulphate at pH 5 followed by total-nitrogen determination on the protein precipitate. These two methods were in close agreement. *Bicarbonate* was estimated by the manometric method of Van Slyke and Neill (1924) on samples equilibrated against human alveolar air (see McDougall 1948); *phosphate* by the micro-method of Allen (1940); *chloride* by the volumetric method of Sendroy (1937); *sodium* and *potassium* by flame photometry; and *calcium* by permanganate titration of the oxalate (A.O.A.C. 1945).

Samples which had to be stored for analysis were kept at -20°C .

TABLE 1. COMPOSITION OF SALIVAS FROM CALF A

The Origin of the Salivary Samples and the Nature of the Stimulation	Nitrogen (mg N/100 ml)			Anions (me./litre)			Cations (me./litre)			
	Total	Urea	Protein	Rest	HCO ₃	HPO ₄ ²⁻	Cl ⁻	Na ⁺	K ⁺	Ca ²⁺
A1. Mixed. From mouth of anaesthetised animal.	15.0	11.1	2.0	1.9	116.6	29.2	7.4	115	17.0	2.5
A2. Parotid. Unstimulated.	16.2	13.3	2.0	0.9	106.5	21.2	5.8	143	11.3	2.8
A3. Parotid. Carbachol.	18.6	14.3	2.5	1.8	117.2	21.6	12.2	150	16.7	3.4
A4. Parotid. Carbachol.	19.0	16.1	1.8	1.1	108.8	19.4	12.8	121	14.7	3.4
A5. Parotid. Rumen pressure.	20.2	16.4	3.5	0.3	105.3	17.8	12.0	123	15.6	2.8
A6. Intermediate. One parotid + one submaxillary + residual.	34.4	12.4	19.0	3.0	77.3	12.4	21.2	103	16.6	4.6
A7. Submaxillary. Unstimulated.	26.8	9.9	11.3	5.6	10.5	0.4	—	—	—	—
A8. Submaxillary. Carbachol.	26.4	10.3	15.0	1.1	17.0	0.2	12.6	13.8	12.9	6.1
A9. Submaxillary. Rumen pressure.	165.2	13.2	90.3*	61.7*	—	—	—	70	15.7	7.7

* Protein was estimated after storage at -20°C . Material had coagulated on the wall of the container and could not be redispersed completely.

RESULTS

The analyses given in Table 1 are for samples obtained from a single calf. Mixed saliva (A1) was collected as it flowed from the mouth while the animal was anaesthetised. The left parotid and submaxillary ducts were then cannulated and the parotid and submaxillary salivas (A2 and A7) collected without stimulation. The quantity of submaxillary saliva secreted was small. Carbachol (5–10 mg/kg body weight) was then injected intravenously and produced a greatly enhanced flow of saliva. Parotid (A3 and A4) and submaxillary (A8) samples were collected. After the effects of the carbachol had disappeared, the rumen of the calf was inflated with nitrogen to a moderate pressure of 5–15 mm Hg and the samples A5 and A9 were collected. The submaxillary saliva obtained after inflation was markedly different from that produced by carbachol and was extremely viscous and cloudy in appearance. Sample A6 was mixed saliva from the mouth, collected after cannulation of the ducts, and represented the sum of secretions from one parotid and one submaxillary gland, together with the whole of the residual saliva, and is a mixture of saliva obtained after stimulation due to both carbachol and inflation of the rumen.

In the second experiment, the results of which are given in Table 2, the conditions were the same as with the previous animal. After collection of the preliminary mixed saliva from the mouth, one parotid and one submaxillary duct were cannulated and saliva samples collected. With calf A, difficulty was encountered in obtaining samples of submaxillary saliva large enough for complete analysis. In the second experiment this was achieved, but the submaxillary saliva samples were derived from somewhat mixed stimulation.

In Table 3, analyses of saliva samples from a third calf are given. The experimental conditions were the same as above except that both parotid and both submaxillary ducts were cannulated and "residual" saliva was collected from the mouth. After collection of the preliminary "mixed" saliva from the mouth and cannulation of the ducts, carbachol was injected and samples C2, C3, and C4 were collected.

When the increased rate of flow had returned to normal, the rumen was inflated. The rates of secretion of the three types of saliva again increased markedly, and samples C5, C6, and C7 were obtained. Rumen pressure was released and carbachol was again injected, giving samples C8, C9, C10. High-frequency stimulation of the sympathetic nerve produced a small quantity of submaxillary saliva, C11, which in appearance and chemical composition was similar to that obtained after inflation of the rumen. Sufficient material was not available for complete analysis, but the sample was characterised by a very high mucoprotein content. The high concentration of urea, which does not appear to be due to analytical error, cannot be explained. The remaining samples C12 and C13 in Table 3, although derived from mixed stimulation, showed that the animal was secreting normal saliva after repeated stimulations.

TABLE 2. COMPOSITION OF SALIVAS FROM CALF B

The Origin of the Saliva and the Nature of the Stimulation	Nitrogen (mg N/100 ml)			Anions (me./litre)			Cations (me./litre)		
	Total	Urea	Protein	Rest	HCO ₃	HPO ₄ ²⁻	Cl ⁻	Na ⁺	K ⁺ Ca ²⁺
B1. Mixed. From mouth of anaesthetised animal.	12.2	9.3	1.5	1.4	121.0	23.0	5.4	154	17.0 2.4
B2. Parotid. Unstimulated.	15.4	12.1	1.0	2.3	104.4	17.8	17.6	134	13.5 2.7
B3. Parotid. Carbachol.	15.6	14.4	1.3	-0.1	99.6	18.8	9.6	150	15.3 6.0
B4. Parotid. Carbachol.	16.4	14.3	1.5	0.6	105.6	14.8	30.9	164	12.8 2.5
B5. Submaxillary. Mixed stimulation.	44.0	10.6	27.4	6.0	18.1	0	17.6	42	16.0 5.1
B6. Submaxillary. Mixed stimulation.	53.0	7.6	34.1	11.3	18.8	0.5	28.6	14.7	14.2 8.5

TABLE 3. COMPOSITION OF SALIVAS FROM CALF C

The Origin of the Saliva and the Nature of the Stimulation	Nitrogen (mg N/100 ml)			Anions (me./litre)			Cations (me./litre)		
	Total	Urea	Protein	Rest	HCO ₃ ⁻	HPO ₄ ²⁻	Cl ⁻	Na ⁺	K ⁺ Ca ⁺⁺
C 1. Mixed. From mouth of anaesthetised animal.	15.6	11.6	1.0	3.0	114.5	21.0	11.9	131	13.8 3.5
C 2. Parotid. Carbachol.	14.6	11.5	1.5	1.6	113.8	22.0	22.1	122	12.2 2.7
C 3. Submaxillary. Carbachol.	29.2	9.0	19.5	0.7	19.6	1.2	22.0	12.9	14.2 10.1
C 4. Residual. Carbachol.	20.4	10.1	9.8	0.5	94.2	19.4	33.0	103	18.2 3.8
C 5. Parotid. Rumen pressure.	14.8	12.5	1.0	1.3	116.3	27.0	9.2	122	13.7 3.5
C 6. Submaxillary. Rumen pressure.	116.8	13.1	96.3	7.4	17.2	2.6	49.2	15.8	14.3 7.6
C 7. Residual. Rumen pressure.	22.8	9.3	12.0	1.5	104.3	17.0	17.5	142	19.4 3.2
C 8. Parotid. Carbachol.	18.0	13.5	2.5	2.0	103.1	27.4	11.0	115	12.2 3.9
C 9. Submaxillary. Carbachol.	26.4	15.9	15.4	--4.9	--	0	58.1	14.2	14.6 5.1
C10. Residual. Carbachol.	22.4	9.7	11.6	1.1	102.5	20.8	64.3	117	15.9 4.7
C11. Submaxillary. Sympathetic nerve stimulation.	127.6	31.4	90.4	5.8	19.9	--	--	--	--
C12. Parotid. Mixed stimulation.	22.4	14.5	2.8	5.1	108.8	22.2	16.9	131	12.8 2.6
C13. Submaxillary. Mixed stimulation.	44.8	7.3	36.7	0.8	18.6	0.1	16.7	13.1	15.1 8.5

Average values for the components of calf salivas are given in Table 4. The results are arranged in two groups, (1) those obtained after carbachol stimulation, and those obtained after inflation of the rumen or electrical stimulation of the distal end of the cervical sympathetic nerve. Where no standard errors are given, there are one or two observations only, and these means must be read with this fact in mind. The number of observations in each set is given in brackets. The observations are spread over three animals and the calculated standard errors include animal variation. No tests of significance have been performed, but differences between any two results of approximately three times the average of the two recorded standard errors would be significant at the 5% level of probability.

TABLE 4. AVERAGE COMPOSITION OF CALF SALIVA STIMULATED BY CARBACHOL OR INFLATION OF THE RUMEN

A. Nitrogen (mg N/100 ml)

Nature of Stimulant			Total N	Urea N	Protein N
Parotid	(1) All samples except rumen pressure	(9)	17.4 $\pm$ 0.80	13.8 $\pm$ 0.46	1.88 $\pm$ 0.06
	(2) Inflation of rumen	(2)	17.5	14.5	2.3
Submaxillary	(1) Carbachol	(3)	27.2 $\pm$ 0.2	11.3 $\pm$ 1.7	15.3 $\pm$ 2.2
	(2) Inflation of rumen	(3)	137 $\pm$ 23	19.3 $\pm$ 7.5	92.3 $\pm$ 2.4
Residual	(1) Carbachol	(2)	21.4	9.9	10.7
	(2) Inflation of rumen	(1)	22.8	9.3	12.0

B. Anions (me./litre)

Nature of Stimulant			Bicarbonate	Phosphate	Chloride
Parotid	(1) All samples except rumen pressure	(9)	107.5 $\pm$ 1.39	20.6 $\pm$ 1.17	15.4 $\pm$ 2.51
	(2) Inflation of rumen	(2)	110.8	22.4	10.6
Submaxillary	(1) Carbachol	(3)	15.7 $\pm$ 2.7	0.46 $\pm$ 0.30	30.9 $\pm$ 14.7
	(2) Inflation of rumen	(3)	18.6 $\pm$ 1.4	2.60	49.2
Residual	(1) Carbachol	(2)	98.4	20.2	48.7
	(2) Inflation of rumen	(1)	104.3	17.0	17.6

C. Cations (me./litre)

Nature of Stimulant			Sodium	Potassium	Calcium
Parotid	(1) All samples except rumen pressure	(9)	136.6 $\pm$ 5.43	13.5 $\pm$ 0.58	3.33 $\pm$ 0.37
	(2) Inflation of rumen	(2)	123	14.7	3.39
Submaxillary	(1) Carbachol	(3)	13.6 $\pm$ 0.5	13.9 $\pm$ 0.8	7.1 $\pm$ 2.0
	(2) Inflation of rumen	(2)	*	15.0	7.7
Residual	(1) Carbachol	(2)	110	17.1	4.3
	(2) Inflation of rumen	(1)	142	19.4	3.2

* The two figures were 70.0 and 15.8.

A comparison was made between the composition of the saliva collected from the calves and that of saliva from the mouth of a mature cow feeding on herbage. The collecting device consisted of a perforated copper tube fitted under the tongue round the lower jaw of the animal (Reid unpublished). On application of suction from a water pump, saliva was readily obtained while the animal was eating and without any apparent contamination from the herbage. From the position of the apparatus in the mouth, it would be expected that this mixed saliva would have a high proportion of the submaxillary and sublingual components. In Fig. 5 the nitrogen analysis of mixed saliva is shown for a single animal fed on fresh red clover over four successive days. Each sample represents a whole day's collection including two 2 h feeding periods.

TABLE 5. NITROGEN ANALYSIS OF MIXED SALIVA FROM THE MOUTH OF AN ANIMAL FEEDING ON RED CLOVER

	1st Day	2nd Day	3rd Day	4th Day	Mean Values
Total N	32.0	32.4	43.4	35.8	35.9 $\pm$ 2.7 mg N/100 ml
Urea N	5.9	6.2	6.7	6.5	6.3 $\pm$ 0.2 mg N/100 ml
Protein N	24.6	25.1	34.7	32.3	29.2 $\pm$ 2.6 mg N/100 ml

It is apparent from the results that the total nitrogen and protein nitrogen are significantly higher than figures obtained for pure submaxillary saliva produced by carbachol. The concentration of urea nitrogen is small. It would appear that normally an appreciable amount of mucus-rich saliva is secreted when the animal is chewing.

DISCUSSION

The results show that the compositions of the three types of saliva analysed, namely parotid, submaxillary, and residual saliva, are very different, and the most striking difference is between parotid and submaxillary saliva. The former contains sodium and bicarbonate in concentrations of about 0.1 to 0.15 M while in the latter these ions are present in concentrations that are 5 to 10 times less. The parotid saliva contains phosphate in concentrations of 0.015 to 0.03 M while the submaxillary saliva contains only traces. The submaxillary saliva contains twice as much calcium as parotid saliva while the concentrations of potassium in both are similar. The concentrations of chloride were erratic with a high standard error.

The results also show that the total nitrogen in submaxillary and residual saliva is significantly higher than in parotid. This was found to be due to large differences in protein nitrogen. The nitrogen of parotid saliva consists mainly of urea which contributes 75–85% of the total nitrogen. Protein nitrogen is present in only small amounts, 10–17% of the total nitrogen, and appears to be a true protein which is readily denatured. Unidentified nitrogen is present only in small amounts.

The protein present in submaxillary and residual salivas is a mucoprotein which was neither heat-denatured nor precipitated by the usual protein precipitants. There were no significant differences in urea nitrogen in the three forms of saliva.

Inflation of the rumen had a very marked effect on submaxillary saliva. It became extremely viscous and cloudy in appearance and the total nitrogen increased sixfold in comparison with that obtained after injection of carbachol. This increase was due largely to an increase in mucoprotein. There was no effect on the urea concentration.

It is important to consider the nature of the stimuli used, especially in relation to the submaxillary glands which are complex structures. The effect of carbachol on the salivary glands is essentially the same as the effect of acetyl choline. Stimulation of the parasympathetic supply to submaxillary glands causes secretion and vasodilation. The same effect is produced by acetyl choline although the dilatory effect may not be so pronounced. Atropine blocks the secretory effect but does not abolish the vasodilatory effect of parasympathetic nerve stimulation. The reason for this has been a matter of some dispute, but recently evidence has been produced (Hilton and Lewis 1955a and b) that the vasodilatory effect is due to the production in the gland of a vasodilatory substance which is not antagonised by atropine. Carbachol, therefore, can be expected not only to cause secretion but also to permit vasodilation, so that the augmented flow of saliva produced is likely to be similar in composition to a true parasympathetic type of secretion.

The effects of sympathetic stimulation are more complex and in the one experiment in which this was performed the scanty viscous saliva produced suggests that in the calf the gland behaves as it does in the dog to sympathetic stimulation, and the concentrated nature of the mucoprotein is partly due to the constrictor effect on the blood vessels which inevitably reduces the volume of the secretion. The fact that inflation of the rumen produced a similar type of saliva is not easy to explain, for the responses of the parotid gland are typically those to be expected from reflex stimulation via the parasympathetic nerve to the gland. It is unfortunate that lack of time did not allow more experiments and analysis of the reflex responses of the submaxillary gland to be made, and further work is needed.

The responses of this gland to feeding vary with the nature and consistency of the food and so it is not surprising that the protein content of the mixed saliva collected from under the tongue, which would be rich in submaxillary and sublingual saliva, does not conform to the samples obtained from anaesthetised calves. Parotid saliva is not affected by anaesthetisation in electrolyte composition and so there is no reason to suppose that submaxillary saliva will be radically altered in this respect. Changes in the mucoprotein content, however, are known to be influenced markedly by a variety of stimuli and the rate of secretion probably exerts the greatest effect.

It is interesting to note that "mixed" saliva obtained from the anaesthetised animal is almost identical in composition with the un-

stimulated parotid saliva. This suggests that the residual saliva is similar to parotid saliva, a fact borne out by the analytical data, except that the concentration of protein nitrogen is greater in residual than parotid saliva.

McDonald (1948) pointed out the importance of salivary urea as a readily available source of nitrogen for rumen organisms and its role in the protein economy of the ruminant. Somers (1957) claimed that in the sheep 65–70% of the salivary nitrogen is in the form of urea and that other nitrogenous compounds are present only in insignificant amounts. The present work on bovine saliva shows that this is true of parotid saliva where urea may account for about 80% of the nitrogen. With residual saliva, however, protein nitrogen is present in amounts as great as that of urea and in the case of submaxillary saliva protein nitrogen may be present at five times the concentration of urea, depending on the type of stimulation. Preliminary experiments (unpublished, this laboratory) in which the concentration of salivary mucoprotein in rumen liquor has been followed during feeding periods, indicate that it is readily metabolised by rumen micro-organisms.

Little information is available on the relative rate of secretion of the different salivary glands but the early work of Ellenberger and Hoffmeister (1887) showed that during mastication submaxillary saliva was secreted at 69% to 100% of the rate of the parotid secretion. In addition, the protein of residual saliva may make a significant contribution. This would suggest that salivary protein can play a similar role to urea in that it can be an "internal" source of nitrogen for the rumen organisms. Analysis of mixed saliva collected from the mouth while the animal is eating shows that protein is present in much larger amounts than is urea. The position of the collecting device in the mouth would tend to withdraw preferentially submaxillary saliva so that the samples obtained are not true mixed saliva. Nevertheless, considering the bulk of the residual saliva and its protein content, it seems that as much as 15 g nitrogen per day in the form of protein may be secreted by an adult cow.

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THE ISOLATION AND CULTIVATION OF SOME FUNGI FROM SOILS AND PASTURES ASSOCIATED WITH FACIAL ECZEMA DISEASE OF SHEEP

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Summary

Some fungi, isolated from soil and pasture grasses, have been examined in investigations relating to facial eczema disease of sheep.

Cultures of fungi were submitted to Ruakura Animal Research Station for the "beaker test", which had frequently been found to give positive results with toxic grass. A positive test was given by a high-sporing strain of *Sporidesmium bakeri* Syd., isolated from pasture grass. Negative tests were given by the other species.

Eleven fungi (six species), including a strain of *S. bakeri*, were grown on modified Czapek-Dox and Raulin-Thom media and fed to guinea pigs. Examination of livers at Wallaceville Animal Research Station showed no symptoms characteristic of the facial eczema toxin associated with grass. A high-sporing strain and two other strains of *S. bakeri* were later cultured on a number of media and the macerated fungal felts and culture fluids were sent to Ruakura Animal Research Station for animal feeding experiments. Some of these cultures produced in the guinea pig symptoms associated with facial eczema disease.

INTRODUCTION

Over the past few years investigations have been carried out in this laboratory on the microbiology of various tussock, pasture, and forest soils and of the vegetation and litter on some of them. A number of groups of micro-organisms has been studied and fluctuations have been recorded both in the total numbers of micro-organisms and in the numbers of particular species, as the result of the influence of climate, vegetation, soil, and other factors. Marked seasonal fluctuations have been recorded in the occurrence of certain species in some investigations (Thornton 1958; Stout 1958; di Menna 1959).

As a result of these studies, it seemed possible that certain micro-organisms, in soil or on pasture plants, might, under suitable climatic conditions, produce a substance causing facial eczema on ingestion by grazing animals. To be effective, any toxin produced by soil micro-organisms would probably have to be taken up by the pasture plants, whereas a toxin produced by micro-organisms on the leaves or litter of these plants could be ingested directly by animals.

Facial eczema is a seasonal disease which affects sheep and cattle grazing on rapidly growing pastures after warm autumn rains and summers with temperatures above average. The disease is most prevalent in North Island districts in New Zealand. An account of the disease is given by Cunningham *et al.* (1942) and a summary of its investigation is given by Filmer (1958).

The possibility of a microbiological cause of facial eczema had previously been considered but it was concluded by Cunningham *et al.* (1942), as a result of earlier investigations, that the disease was not due to infection by a specific bacterial agent. Some of the endophytic fungi of various pasture grasses had been considered by Neill (1940) and Cunningham (1958a) but no evidence of their toxicity for farm animals had been found. Simpson *et al.* (1957) stated that "Careful examination of paddocks in which facial eczema had developed failed to discover any weed or fungus which could be incriminated".

To examine the possibility that micro-organisms cause facial eczema, it was considered essential to study areas with a known history of the disease. Such areas were being investigated by a team of workers of the Department of Agriculture who were taking regular samples of grass for toxicity tests. The Department of Agriculture kindly offered us the use of these areas and co-operative studies were begun with Ruakura Animal Research Station, Hamilton, in February 1957, at Claudelands Showgrounds, Hamilton. This account records the isolation and cultivation of fungi from soil and pasture grass in some of these areas and also in an area at Marton, which was being simultaneously examined as part of a broader investigation, by this laboratory, of forest and pasture soil microbiology. Several strains of *Sporidesmium bakeri* have been isolated from pastures in these areas and also from an area maintained by the Plant Chemistry Division, Department of Scientific and Industrial Research, at Palmerston North. A high-sporing strain, C, has been disposed by the Commonwealth Mycological Institute, Kew, England, as *Sporidesmium bakeri* Syd. (Hughes 1953).*

Cultures of several of the predominant fungi have been submitted to Ruakura Animal Research Station for "beaker tests". The "beaker test" had been shown by Perrin (1959) and Sandos *et al.* (1959) to be associated frequently with samples of toxic grass. Cultures have also been fed to guinea pigs at our laboratory and at Ruakura and Wallaceville Animal Research Stations to test for the presence of toxin.

During the course of this investigation, it was reported in a discussion by Dr K. J. Mitchell (see Butler and Rae 1957) that, in an exploratory trial, control of facial eczema was effected by the application of copper sulphate at high rates to pasture. This could have been due to the action of copper on the microflora of the soil.

MATERIALS AND METHODS

Sources of Samples

Samples were taken from pastures dominated by ryegrass and white clover mainly from areas in which facial eczema had occurred recently or with some degree of regularity in the past. These areas were located at the polo field, Claudelands Showgrounds, Hamilton.

* Recently Hughes (1958) has proposed the new genus *Scheleobracea* and has indicated that *Sporidesmium bakeri* Syd. is a facultative synonym for *Scheleobracea echinulata* (Speg.) Hughes.

Mr F. Hardy's farm, near Hamilton, Mr D'Arcy Smith's farm, near Matamata, and Mr R. Stafford's farm near Marton. Turfs from Gisborne Racecourse, maintained at Grasslands Division, Palmerston North, were also sampled.

Soil and pasture were also sampled from an experimental plot of perennial ryegrass maintained by the Plant Chemistry Division, Department of Scientific and Industrial Research, on Massey College farm, Palmerston North (Butler 1958).

Isolation of Fungi

Fungi were isolated from soil by the screened immersion plate method (Thornton 1952) from a depth of 2–3 in. at Mr R. Stafford's farm near Marton and 1–2 in. at Claudelands Showgrounds, at Mr F. Hardy's farm near Hamilton, and in turfs from Gisborne Racecourse.

Hyphae growing over pasture grasses were picked off with sterilised watchmaker's tweezers and transferred to culture plates of potato-glucose agar, incubated, and subsequently examined.

Material dusted off the gang-mower at Claudelands Showgrounds and sent by Mr J. C. Percival was taken from the container with a sterilised needle and lightly distributed over the surface of sterile potato-glucose agar culture plates, and examined after incubation.

Repeated subculturing of the growths obtained resulted in the establishment of pure cultures.

Spore Counts

Estimation of numbers of spores of *S. bakeri* on grass samples was carried out as follows: Freeze-dried grass (5 g supplied by Dr G. W. Butler) was macerated in 200 ml water for 1 min and then filtered through a 2 mm sieve. Counts were carried out with a haemocytometer on twelve separate portions of each sample.

Media

The following media have been used for the growth of fungi. The media (200 ml) were distributed in conical flasks (1 l) and sterilised at 15 lb/in.² for 15 min unless otherwise stated.

(1) *Potato-glucose agar*: Washed, chopped potatoes (200 g) were steamed for 30 min with tap water (1 l) and strained through coarse muslin. Glucose (20 g/l) and agar (15 g/l) were added to the filtrate and the medium adjusted to *ca.* pH 5.5.

(2) *Potato-carrot extract medium*: Washed potatoes (200 g) and carrots (200 g) were cut into small pieces without peeling and boiled with tap water (1 l) for 30 min. The extract was strained through coarse muslin. As a normal procedure, the extract was used without the addition of other compounds.

In preliminary experiments, extracts were also prepared at a concentration of 20, 50, and 100 g/l of each of potatoes and carrots, and to some of the media salts were added as follows: NaNO₃, 0.2 g; KH₂PO₄, 0.1 g; glucose, 20 g/l.

(3) (i) *Aqueous macerated grass extract medium*: Dried non-toxic grass (100 g) was macerated, in portions, with tap water (1 l) in a Townson and Mercer "Top Drive Macerator". The resulting thick suspension was squeezed through a muslin bag and the filtrate centrifuged for 30 min at 1000 g. The clear supernatant liquid (650 ml) was diluted with tap water to 1 l and then sterilised by filtration through a Seitz filter.

(ii) *Aqueous macerated grass extract medium plus glucose*: The following compounds were used as a supplement to the above grass extract medium, viz. $(\text{NH}_4)_2\text{SO}_4$, 0.2 g; KH_2PO_4 , 0.1 g; glucose, 20 g/l.

Both grass extract media (100 ml) were distributed in conical flasks (500 ml).

(4) *Casamino acid-glucose medium*: KCl , 0.5 g; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.5 g; KH_2PO_4 , 1.0 g; $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, 0.01 g; casamino acids, 3.5 g; glucosamine hydrochloride, 0.5 g; yeast extract, 0.1 g; glucose, 30 g; pH 6.3. The glucose was added to the medium after separate sterilisation.

(5) *Czapek-Dox medium*: NaNO_3 , 2.0 g; KCl , 0.5 g; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.5 g; KH_2PO_4 , 1.0 g; $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, 0.01 g; glucose, 50 g; distilled water, 1 l; pH 6.0.

(6) *Raulin-Thom medium*: Ammonium tartrate, 2.67 g; $(\text{NH}_4)_2\text{HPO}_4$, 0.40 g; $(\text{NH}_4)_2\text{SO}_4$, 0.17 g; K_2CO_3 , 0.40 g; MgCO_3 , 0.27 g; $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, 0.05 g; $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$, 0.05 g; tartaric acid, 2.67 g; glucose, 50 g; pH, 6.0.

(7) *Modified Raulin-Thom-sucrose medium*: As (6) above but with sucrose (30 g/l) replacing glucose and the following growth factors added: thiamin, 1 p.p.m.; nicotinic acid, 1 p.p.m.; riboflavin, 1 p.p.m.; calcium pantothenate, 0.5 p.p.m.; pyridoxine, 0.5 p.p.m.; biotin, 0.2 p.p.m.; p-aminobenzoic acid, 0.1 p.p.m.

(8) *Modified Fries No. 3 basal medium* (cf. Pringle and Braun 1957). Ammonium tartrate, 5.0 g; NH_4NO_3 , 1.0 g; KH_2PO_4 , 1.0 g; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.50 g; NaCl , 0.1 g; $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, 0.13 g; $\text{MnSO}_4 \cdot 4\text{H}_2\text{O}$, 1 mg; boric acid, 1 mg; $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$, 0.1 mg; $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$, 0.1 mg; $\text{FeSO}_4 \cdot 6\text{H}_2\text{O}$, 20 mg; sucrose, 20 g; distilled water, 1 l.

The medium (100 ml) was distributed in conical flasks (1 l).

Inocula

Aqueous suspensions of spores and/or mycelium were employed to inoculate flasks.

In the cultivation of *S. bakeri*, aqueous suspensions of spores and mycelia from cultures grown on 200 ml of potato-carrot extract agar (surface area 125 sq. cm) were prepared by gently rubbing the surface growth with a sterile platinum wire. This suspension was used for inoculating *ca.* 15 culture flasks.

Incubation

Cultures were incubated without shaking at 24°C in the dark unless otherwise stated,

"Beaker Tests"

All fungal samples for "beaker tests" were submitted to Ruakura Animal Research Station.

"Beaker tests" on samples of perennial ryegrass from Palmerston North were carried out by Plant Chemistry Division.

Animal Feeding Experiments

After incubation, the mycelial felts were macerated with the culture fluids and stored in the dark at *ca.* -20°C . This material was fed to guinea pigs as it had been shown by Evans *et al.* (1957) and Perrin (1957) that this animal could be used to indicate the presence of the facial eczema toxin in grass. Feeding experiments were as follows:

(a) *Fungi other than Sporidesmium bakeri*

For the feeding experiments shown in Table 2, fungi were grown on Czapek-Dox and Raulin-Thom media with and without yeast extract (1.0 g/l). The macerated fungal material was mixed with meal (cf. Cunningham (1958b) for formula) and molasses to which gelatin had been added to make a firm cake. Sufficient was fed to guinea pigs to provide a daily portion of 150–200 mg dry weight mycelium.

(b) *Sporidesmium bakeri*

In initial experiments with *S. bakeri*, strain CL, the above procedure was adopted and the material was fed to guinea pigs by Mrs G. Lawson at Wallaceville Animal Research Station.

Pathological examinations of guinea pig livers, after the above two series of feeding experiments, were made by Mr W. J. Hartley of Wallaceville Animal Research Station.

Other strains of *S. bakeri* were grown as shown in Table 5 and submitted to Mr J. C. Percival at Ruakura Animal Research Station for animal feeding experiments (Percival 1959).

Further material of *S. bakeri*, grown on the potato-carrot extract medium, was sent to Ruakura Animal Research Station for use in other animal feeding experiments.

RESULTS

Fungi Other than *Sporidesmium bakeri**Isolation*

(a) *Soil*: Approximately four hundred and eighty fungi were isolated from the soil samples examined. *Fusarium oxysporum* was the predominant fungus, particularly in February and March, and *Fusarium culmorum* and *Trichoderma viride* were also commonly present (Table 1). An account of the fungi more frequently isolated from the soil at Marton has been given by Thornton (in press).

(b) *Hyphae on Grass*. Fungal hyphae were not observed in an examination of pasture at Claudelands Showgrounds and Mr F. Hardy's farm in March 1957. A sample of hyphae on grass, from an

experimental area at Matamata, was later sent by Mr J. C. Percival, in May 1957 and one fungus only, *Sclerotinia trifoliorum*, was isolated.

Chaetomium sp. was the predominant fungus growing on grass from turfs taken from Gisborne Racecourse and maintained in a glass-house by Dr K. J. Mitchell under warm humid conditions (Table 1).

As the above species were predominant in the samples examined they were employed in further investigations.

TABLE 1. FUNGI ISOLATED FROM SOIL AND GRASS SAMPLES

Samples		Fungi
Date coll.	Type of sample and location	
Oct 56 Feb 57 May 57	Soil fungi from under pasture, Marton.	<i>Fusarium oxysporum</i> , <i>Trichoderma viride</i> , and <i>Fusarium culmorum</i> predominant
Feb 57 Mar 57	Soil fungi from under pasture, Claudelands Showgrounds, Hamilton.	<i>Fusarium oxysporum</i> predominant
Mar 57	Soil fungi from under pasture, Mr. Hardy's Farm.	<i>Fusarium oxysporum</i> predominant
Apr 57	Hyphae on grass, Matamata.	<i>Sclerotinia trifoliorum</i>
Aug 57	Hyphae on grass on turfs from Gisborne Racecourse placed in warm, humid glasshouse at Grasslands Division, Palmerston North.	<i>Chaetomium</i> sp. <i>Mucor</i> spp. <i>Fusarium</i> sp. <i>Botrytis</i> sp.
	Soil fungi from turfs from Gisborne, maintained in the open at Palmerston North.	<i>Fusarium culmorum</i> , and <i>Fusarium oxysporum</i> predominant
Mar 58	Hyphae on grass, Claudelands Showgrounds, Hamilton.	<i>Sporidesmium bakeri</i> <i>Fusarium</i> sp. <i>Mucor</i> sp. <i>Epicoccum</i> sp. <i>Absidia</i> sp. <i>Marasmius graminum</i> (as sporophore)
Mar 58	Dustings from gang-mower, Claudelands Showgrounds, Hamilton.	<i>Sporidesmium bakeri</i>
Apr 58	Hyphae on dead grass, Massey College, Palmerston North.	<i>Sporidesmium bakeri</i> <i>Fusarium</i> sp.

Animal Feeding Experiments

Several of the fungi isolated were grown as shown in Table 2 and fed to guinea pigs for 32 days. After this period the guinea pigs were killed and examined by Mr W. J. Hartley. There were no symptoms characteristic of those produced by the facial eczema hepatotoxin.

TABLE 2. CULTIVATION OF SOME SOIL AND GRASS FUNGI FOR ANIMAL FEEDING EXPERIMENTS

Fungus	Source of Fungus	Strain (Herb. I.M.I. No.)	Medium	Incubation time, days
<i>Fusarium oxysporum</i> (4 strains)	Soil, Claudelands Showgrounds, Hamilton	71580	*Czapek-Dox + Zn (0.4 p.p.m.) *Raulin-Thom	14 and 22 22
		71581	*Czapek-Dox + Zn (0.4 p.p.m.) *Raulin-Thom	14 and 22 22
	Soil, Marton	71582	*Czapek-Dox + Zn (0.4 p.p.m.)	14 and 22
		71584	*Czapek-Dox + Zn (0.4 p.p.m.)	14 and 22
<i>Fusarium culmorum</i>	Soil, Marton	71578	*Raulin-Thom	18 and 22
<i>Trichoderma viride</i> (3 strains)	Soil, Marton	—	Raulin-Thom + yeast extract (0.1%) (The 3 strains were grown and fed separately)	12
<i>Sclerotinia trifoliorum</i>	Grass, Matamata	—	Raulin-Thom + yeast extract (0.1%)	20
<i>Chaetomium</i> sp.	Grass, turfs from Gisborne Race- course maintained at Palmerston Nth.	—	Raulin-Thom	6

* These media were adjusted to *ca.* pH 4.0 before sterilisation.

"Beaker Tests"

Several soil and pasture fungi were grown on sterilised non-toxic grass (cut pasture) and ryegrass roots. The fungi grew well on these media and *Fusarium culmorum* grew particularly vigorously on the sterilised ryegrass roots. After the incubation periods indicated in Table 3, these cultures were submitted to Ruakura Animal Research Station for "beaker tests". The results of all tests were negative.

TABLE 3. CULTIVATION OF SOME SOIL AND GRASS FUNGI FOR "BEAKER TESTS"*

Inoculum	Source	Medium (incubation time, days)	Date of Cultivation
1 <i>Fusarium oxysporum</i>	Soil, Claudelands Showgrounds, Hamilton	Sterilised non-toxic grass, 25 g (3,5,7)	Dec, 1957
2 <i>Fusarium culmorum</i>	"	"	Dec, 1957
3 <i>Trichoderma viride</i>	"	"	Dec, 1957
4 <i>Sclerotinia trifoliorum</i>	Grass, Matamata	"	Dec, 1957
5 <i>Chaetomium</i> sp.	Grass, Gisborne turfs	"	Dec, 1957
6 <i>Fusarium oxysporum</i>	Soil, Claudelands Showgrounds	Sterilised ryegrass roots, 75 g (42)	Mar-Apr, 1958
7 <i>Fusarium culmorum</i>	"	"	Mar-Apr, 1958
8 <i>Trichoderma viride</i>	"	"	Mar-Apr, 1958
9 <i>Fusarium oxysporum</i>	Soil, Palmerston North	Sterilised non-toxic grass, 25 g (17)	May, 1958
10 <i>Fusarium culmorum</i>	"	"	May, 1958
11 <i>Trichoderma viride</i>	"	"	May, 1958
12 <i>Sclerotinia trifoliorum</i>	Grass, Matamata	"	May, 1958
13 <i>Chaetomium</i> sp.	Grass, Gisborne turfs	"	May, 1958
14 <i>Sporidesium bakeri</i> (strain CL)	Grass, Claudelands Showgrounds	"	May, 1958
15 <i>Marasmius graminum</i>	"	"	May, 1958
16 Mixture of 9-15 above	—	"	May, 1958
17 Dustings from gang-mower	"	"	May, 1958
18 "Beaker test" positive grass	Palmerston North	"	May, 1958
19 <i>Mucor</i> sp.	"Beaker test," positive grass, Palmerston North.	"	May, 1958

* "Beaker tests" conducted by Ruakura Animal Research Station.

Antibiotic Activity

To gain some indication of the possible production of biologically active metabolites by the fungi, culture solutions were tested for their antibiotic activity, after the growth periods indicated in Table 2; against *Botrytis allii*, *Staphylococcus aureus*, and *Bacterium coli*.

Culture fluids of *Fusarium oxysporum*, *Trichoderma viride*, *Sclerotinia trifoliorum*, and *Chaetomium* sp. showed slight inhibition of the germination of the spores of the fungus *Botrytis allii*, by the technique of Brian and Hemming (1945). Only the culture fluid of *Sclerotinia trifoliorum* inhibited growth of *Staphylococcus aureus*. No species was active against *Bacterium coli*.

Sporidesmium bakeri Syd.

Isolation of Strains

Four samples of hyphae on grass were collected from the polo field, Claudelands Showgrounds, on 15 March 1958, by Mr J. C. Percival and sent for examination. A number of cultures of fungi were obtained from these samples (Table 1) in which *S. bakeri* was later found to be the predominant species. Of the six cultures of *S. bakeri* isolated from the hyphal material, the culture which produced the most spores, strain CL, was selected for initial study. A high-sporing strain, now known as strain C, was later isolated from cultures obtained from this hyphal material on 1 July 1958, and has been used for most investigations. Strain C has been disposed as *Sporidesmium bakeri* Syd. (Herb. I.M.I. No. 74473) by the Commonwealth Mycological Institute, Kew, England.

A sample of mower dustings, suspected to contain black spores, was received from Mr Percival who had obtained it, on 24 March 1958, from a gang-mower used on Claudelands Showgrounds. This material, from which strain BS has been isolated, was found to contain predominantly spores which resembled those of *S. bakeri*. The dustings were later reported (Mr J. C. Percival, pers. comm.) to contain the "beaker test" substance.

S. bakeri, strain G, was isolated from a sample of dead ryegrass, giving a positive "beaker test", collected by Dr G. W. Butler from the experimental plot at Palmerston North on 24 April 1958.

Spore Counts

Counts of spores of *S. bakeri* have been made on samples of ryegrass cut from the plot at Palmerston North by Dr Butler, freeze-dried, and sent for examination in May 1958. These samples contained both green and dead yellowed leaves. Sheep grazing on this area had shown clinical symptoms of facial eczema on 12 March 1958 (Dr G. W. Butler, pers. comm.). Two of these samples had been collected on 13 and 19 March and gave a positive "beaker test". These two samples contained about 13×10^5 spores/g freeze-dried grass, whereas a sample which had been collected on 27 March and gave a negative "beaker test" contained only 0.7×10^5 spores/g freeze-dried grass. Spores were more abundant on dead than on green leaves.

The occurrence of spores of *S. bakeri* on a dead ryegrass leaf is shown in Fig. 1.

Initial "Beaker Tests"

Strain CL was grown on Czapek-Dox and Raulin-Thom media in which growth was stimulated by the addition of yeast extract (1 g/l).

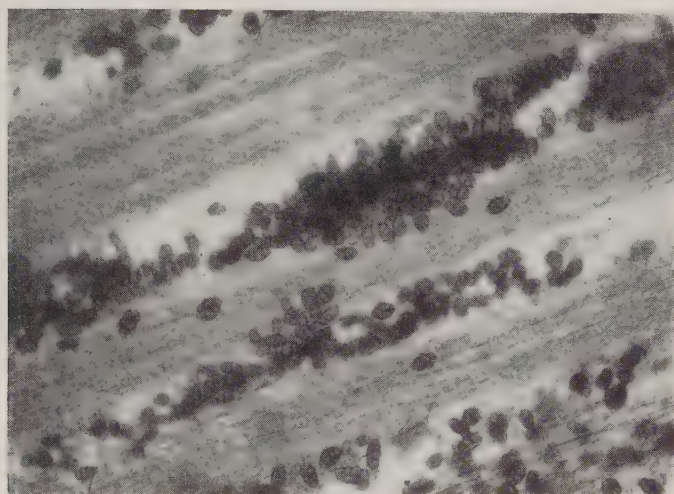


Fig. 1.—Spores of *S. bakeri* on a dead ryegrass leaf.

Photo: R. R. Julian

Felts harvested after incubation on both media for 14 and 21 days were macroscopically free from spores and gave a negative "beaker test". Cultures of strain CL grown on sterilised non-toxic grass (Table 3) also gave negative "beaker tests".

Initial Animal Feeding Experiments

Cultures of *S. bakeri*, strain CL, from equal numbers of flasks of each of the Czapek-Dox and Raulin-Thom media, with and without yeast extract, were harvested on 27 May 1958 after incubation for 21 days, and fed to guinea pigs. The animals were killed after three weeks and the livers examined by Mr W. J. Hartley at Wallaceville Animal Research Station. No liver damage characteristic of facial eczema was recorded.

CULTIVATION OF HIGH-SPORING STRAIN, C

Percival (1959) had found that the spore-containing sample of dustings from the gang-mower at Claudelands gave a positive "beaker test", which Perrin (1959) and Sandos *et al.* (1959) had shown to be associated frequently with toxicity. Large numbers of spores had also been found on samples of dead ryegrass from the experimental plot at Palmerston North and were also apparently present on grass at Claudelands Showgrounds (Percival and Thornton 1958) at toxic periods. For these reasons, the high-sporing strain C was used in additional experiments following its isolation on 1 July 1958.

A number of modifications of the potato-carrot extract and aqueous macerated grass extract media were then employed to see if the sporing capacity of strain C could be further enhanced. Results are given in Table 4. The addition of the salts and glucose to both media (see Methods, media 2 and 3) resulted in stimulation of growth and spore

production, but the stimulation was only slight in the most concentrated media. Sporulation was greatest in the potato-carrot extract (200 g/l of each) medium. In this concentrated potato-carrot extract medium and in the grass extract (100 g/l) medium, inoculated on 9 July 1958, the yields of dry mycelium were respectively 9.0 and 7.5 g/l after incubation for 14 days.

TABLE 4. GROWTH AND SPORULATION OF *Sporidesmium bakeri*, STRAIN C

Medium	Concentration (material extracted, g/l)	Incubation	Growth	Sporulation
Potato-carrot extract	20	7	Poor	Profuse
	50	7	Poor	Profuse
	100	7	Moderate	Profuse
	200	7	Good	Profuse
Aqueous macerated grass extract	10	5	Poor	Weak
	20	5	Poor	Weak
	100	5	Moderate-good	Weak
	100	14	Good	Good

Media were inoculated on 9 July 1958.

A comparison was also made of growth on potato-carrot extract (200 g/l of each) agar, potato extract (200 g/l)-glucose (10 g/l) agar, and malt extract (20 g/l) agar. Growth was good on all media but spore production appeared to be greatest on the potato-carrot extract medium.

On the defined Fries medium, used by Pringle and Braun (1957) for the production of toxin by *Helminthosporium victoriae*, growth was not as uniform, and only one of the four flasks cultured gave many spores after incubation for 10 days.

As the concentrated potato-carrot extract medium was easy to prepare, favoured uniform rapid growth, and gave a good yield of mycelium with copious spore production, it was selected for further studies on the toxicity of the fungus.

"Beaker Tests"

Spores scraped off a culture of strain C on the potato-carrot extract agar medium gave a positive "beaker test" on 9 July 1958.

Positive "beaker tests" were also produced by the heavily-sporing mycelial felts of strain C grown on aqueous macerated grass extract for 14 days, and on Fries medium for 10 days (only heavily-sporing flask tested).

Animal Feeding Experiments

S. bakeri, strain C, was grown on potato-carrot extract medium, inoculated on 10 July 1958, and incubated for 11 days. The mycelial felts and culture fluids were macerated and sent to Ruakura Animal Research Station on 22 July 1958 for animal feeding experiments. This material was shown to be toxic (Percival 1959).

Other more-reproducible media have also been tested for their ability to promote the growth of the fungus and the production of toxin (Table 5). The three strains grew well on the casamino-acid glucose medium but strain G did not appear to produce spores after incubation for 21 days. Growth was slow on the Raulin-Thom-sucrose medium and even after incubation for 21 days, the mycelial felts of strains BS and G had not completely covered the surface of the culture medium.

TABLE 5. CULTIVATION OF *S. bakeri* FOR ANIMAL FEEDING EXPERIMENTS

Medium	Strain	Date of Inoculation, 1958	Incubation, days	Sporulation	Results of animal feeding experiments
Potato-carrot extract	C	10 Jul	11	Heavy	Positive
Casamino acid-glucose	C	18 Jul	4-21*	Moderate	Positive
	BS**	1 Aug	4-21*	Poor	Negative
	G	1 Aug	4-21*	None	Negative
Modified Raulin-Thom-sucrose	C	18 Jul	4-21*	Poor	Negative
	BS**	1 Aug	4-21*	Poor	Positive
	G	1 Aug	4-21*	None	Negative

* Material was harvested from the same number of flasks incubated for 4, 7, 11, 14, and 21 days and bulked before feeding to animals.

** Cultures incubated in the light.

All feeding experiments were conducted by Ruakura Animal Research Station and results were kindly supplied by Mr. J. C. Percival. "Positive" = culture reported as causing development of symptoms characteristic of those produced by the facial eczema toxin.

As the influence of incubation period on toxin production was not known, cultures on some media were incubated for various periods and the material from the same number of flasks was bulked before sending for feeding experiments. All the above cultures were tested for toxicity at Ruakura Animal Research Station. Details of their assay are given by Percival (1959) and a summary of his results is given in Table 5. Cultures of strain C shown to be toxic were grown in the dark and the toxic culture of strain BS on the Raulin-Thom-sucrose medium was incubated in the light. Toxin can, therefore, be produced either in the dark or in the light. It is clear that the composition of the medium affects the production of toxin. Toxin is produced in the two non-

defined media containing organic nitrogen compounds, viz. potato-carrot extract, and casamino acid-glucose, and it can also be produced in a defined medium containing inorganic nitrogen, e.g. by strain BS in the Raulin-Thom-sucrose medium. All cultures which were shown to be toxic contained spores. It is apparent that strains differ in their production of toxin but it is, as yet, uncertain whether the ability of a strain to produce toxin is dependent on its ability to produce spores.

DISCUSSION

Several fungi have been isolated from soil and grass in areas and under seasonal conditions normally associated with the facial eczema disease. Under present cultural conditions *S. bakeri* has been found to produce a toxin which, in guinea pigs, gives symptoms characteristic of facial eczema (Anon. 1958; Thornton and Percival 1959; Percival 1959).

As *S. bakeri* has been isolated from turfs containing hyphae visible in the field and has been observed as spores in large numbers on grass, at times when the grass was either toxic or gave a positive "beaker test", it is suggested that this fungus is a cause of the disease in the field. The small number of samples examined, however, is insufficient to demonstrate its general field incidence and further work is required to determine the exact role of *S. bakeri* in the epidemiology of the disease.

It is apparent that not all strains so far isolated from the field sporulate in culture as readily as strain C. The fact that this high-sporing strain gave a positive "beaker test" (Percival and Thornton 1958) stimulated the examination of this strain for the production of a toxin. The subsequent demonstration of toxicity may indicate that sporulation is associated with the "beaker test" substance and with toxicity. Further work is being undertaken to establish the relationship between the growth stages of the fungus, and the influence of media and cultural conditions, on toxin production.

The demonstration by Percival (1959) that ingestion by animals of toxic cultures of *S. bakeri* causes symptoms characteristic of facial eczema may indicate that the ingestion of metabolic products of other pasture micro-organisms influences animal health. Some of the soil and grass fungi examined in this study may possibly cause other ill-health problems in farm animals.

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TOMATO YIELD TRIALS, 1958-59

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Summary

Trials were carried out on the effect of high-pressure mercury-vapour (h.p.m.v.) illumination of seedlings, pre-planting treatments, and variety upon tomato yields.

H.P.M.V. gave a slight but not statistically significant improvement in early yield. Climatological aspects which affect this result are discussed. Pricking off seedlings into 3½ inch pots gave a highly significant increase in early yield compared with pricking off into wood-vener plant frames. Early and late plantings which were sown and pricked off together gave the same season of cropping and the same yield. However, late planting decreased the early yield and increased the late yield, giving a reduced financial return. The new leaf-mould-resistant variety, V. 548, gave a highly significant increase in yield over Potentate.

INTRODUCTION

The use of high-pressure mercury-vapour illumination for seedlings is well established as a commercial method in countries where natural winter light is low (Lawrence and Calvert 1954). The extra illumination induces more rapid growth and flower initiation, and consequently increases the more financially remunerative early crop. Whether the winter light in New Zealand is sufficiently low to make the method worthwhile has not been ascertained and the experiment reported is a pilot trial for the Canterbury area.

Lawrence (1948) reports the beneficial effects of raising tomato seedlings in larger-sized pots. Very many New Zealand commercial growers raise their plants to planting-out stage in plant frames (Gillard 1952) or even in seed boxes, with apparently satisfactory results. It was felt that this practice, contrary to research findings, should be investigated to see if these English findings could be applied to New Zealand practice.

V. 548 was introduced by the Department of Scientific and Industrial Research from Vineland Experiment Station, Ontario, Canada, because of its immunity to leaf mould (*Cladosporium fulvum*). The trial reported is a yield trial only, comparing it with Potentate, the present standard tomato variety.

METHOD

The variety Potentate was used in all trials except where V. 548 is specifically mentioned. Seed was sown on 19 June 1958, and pricked off into John Innes potting compost No. 2 between 4 and 6 July 1958. Pricking out was into 3½-in. pots, except for plants required in plant

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frames. Plant frames are strips of wood veneer, folded to make a container without top or bottom and then placed in seed trays (Gillard 1952). The size used, 11 in. by 3 in., gave a container $2\frac{1}{2}$ in. square by 3 in. deep when folded, and these were placed 35 to a standard seed tray (15 × 20 in.). The actual soil content of a plant frame was $8\frac{1}{2}$ ounces, compared with $13\frac{1}{2}$ ounces held in a $3\frac{1}{2}$ inch pot. Seedlings were grown on to planting out size in the same house.

Equipment used for h.p.m.v. illumination was a 400 watt, hard-glass, vertical lamp. The horizontal lamp described by Lawrence and Calvert (1954) was not at the time available in New Zealand. The lamp was controlled by a time switch, operating from 12 midnight to 12 midday. The temperature under the lamp in trial runs was 6° to 8° F higher than the rest of the house, so the seedlings and equipment were moved to a cooler house and a small fan used to facilitate air movement. Temperatures then obtained under the lamp were comparable to the temperatures amongst the control seedlings. The average minimum temperature during the period of illumination (24 July to 14 August) was 51° F under the lamp and 50° F amongst control plants.

Two houses were used for planting. House 1, which contained the h.p.m.v. trial, was planted 26 August, 1958, and House 2, which contained the Potentate - V. 548 trial and Plant frames-Pot trial, was planted on 12 September 1958. This latter planting was much later than desirable, owing to circumstances outside our control and some plants were showing signs of check when planted.

Planting in all trials was in two-row beds, which contained one row of each treatment being compared. To avoid possible interaction due to heating and aspect (the houses run north-south and the rows east-west), the positions of the treatments were randomised within all replications. The h.p.m.v. trial consisted of eight replications of rows of eight plants, the plant-frame trial seven replications of rows of ten plants, and the V. 548 trial four replications of rows of ten plants.

Picking was carried out as required throughout the season, both houses being cleared completely of ripe fruit on each picking. Trials were picked first and recorded, the rest of the house was then picked and bulked with the trial lots, and the total yield for the house was recorded.

RESULTS

Yield results are summarised in Tables 1, 2, and 3. The figures were split into monthly or half-monthly totals to show up any tendency to early cropping. Owing to the collapse of a supporting wire in House 1, six plants in the h.p.m.v. trial snapped, and this trial has therefore been calculated on a single-plant basis. The total yields from Houses 1 and 2 were used to assess the effect of delayed planting upon yield, these results being summarised in Table 4. The results were obtained by subtracting the yield for treated plants from the bulk yield and obtaining an average for the remaining plants (House 1: $400-64 = 336$; House 2: $560 - (70 + 40) = 450$).

TABLE 1. EFFECT OF HIGH-PRESSURE MERCURY-VAPOUR ILLUMINATION ON YIELD
(Average yield per plant, in ounces)

		T—Treated C—Control				
Replication		November	December 1-15	December 16-31	January & February	Total
1	T	18.1	55.3	58.0	40.2	171.6
1	C	13.5	57.6	73.0	53.9	198.0
* 2	T	10.4	74.0	77.7	42.0	204.1
2	C	20.3	63.5	65.7	38.4	187.9
3	T	15.9	70.0	51.9	48.0	187.8
* 3	C	19.4	66.6	70.0	50.6	206.6
4	T	17.4	57.0	90.9	52.9	218.2
* 4	C	9.1	65.7	80.7	52.6	208.1
* 5	T	18.9	79.3	79.0	41.6	218.8
5	C	17.1	57.1	51.9	31.5	157.6
6	T	20.2	61.2	60.1	54.7	196.2
6	C	21.4	61.5	74.1	40.7	197.7
* 7	T	28.1	68.4	72.7	52.3	221.5
7	C	23.0	68.4	72.9	58.1	218.4
* 8	T	23.3	67.7	74.1	38.1	203.2
8	C	17.6	56.8	75.9	40.0	195.3
Total	T	152.3	534.9	564.4	369.8	1621.4
Total	C	141.4	493.2	562.2	372.8	1569.6

* These results averaged from seven plants. Other replications averaged from eight plants.

DISCUSSION AND CONCLUSIONS

H.P.M.V. Illumination Trial. The results per plant may be summarised from Table 1 as follows:

		Nov.	Dec. 1-15	Dec. 16-31	Jan. & Feb.	Total
Increase in ounces	H.P.M.V.	+ 1.36	+ 5.21	+ 0.27		+ 6.46
	Control				+ 0.38	

This shows a slight but not statistically significant improvement in yield, which occurs almost entirely in the first month of the trial. This trend in yield is that which would be expected from the work of Lawrence and Calvert (1954). However, the inconsistencies which

TABLE 2. EFFECT OF PRE-PLANTING TREATMENT ON YIELD
(Yield in ounces per ten-plant plot)

PF—Seedlings pricked out into plant frames.

P —Seedlings pricked out into pots.

Replica- tion	November	December 1-15	December 16-31	January	February	Total
1 PF	45	315	652	676	145	1833
1 P	186	277	444	609	168	1684
2 PF	71	231	582	620	181	1685
2 P	110	303	490	719	140	1762
3 PF	91	322	497	742	180	1832
3 P	147	239	460	587	194	1627
4 PF	54	278	612	681	118	1743
4 P	94	244	461	710	210	1719
5 PF	54	201	542	667	131	1595
5 P	159	238	598	643	204	1842
6 PF	39	255	665	712	150	1821
6 P	129	238	609	827	139	1942
7 PF	105	244	682	555	167	1753
7 P	120	297	551	783	219	1970
Total PF	459	1846	4232*	4653	1072	12262
Total P	945**	1836	3613	4878	1274	12546

* Significant at 5% level.

**Significant at 1% level.

4 PF results were recorded on nine plants only. These results were adjusted to ten for statistical analysis.

appear in the Table between yields of compared plots do not suggest that this trend is a very definite one.

The effect of h.p.m.v. upon seedling growth is quite striking and Fig. 1, taken 18 August, shows the difference obtained between treated and control plants. The plants illustrated were representative samples. The larger size and the darker green, more robust appearance of the treated plants were, however, soon equalled by the controls after planting out. When cropping commenced in mid November, no size difference was discernible and there were no obvious differences in truss size and numbers.

It would appear that although illumination proved a distinct advantage in the early months, subsequent natural illumination was

TABLE 3. COMPARISON OF YIELD, POTENTATE AND V. 548
(Yield in ounces per ten-plant plot)

V—V. 548

P—Potentate

Replica- tion	November	December 1-15	December 16-31	January	February	Total
1 V	117	258	732	711	232	2050
1 P	129	296	491	610	101	1627
2 V	184	302	741	964	163	2354
2 P	135	252	510	655	163	1715
3 V	141	297	667	807	204	2116
3 P	168	188	528	577	205	1666
4 V	204	342	622	756	262	2186
4 P	156	279	530	601	167	1733
Total V	646	1199	2762**	3238*	861	8706**
Total P	588	1015	2059	2443	636	6741

* Significant at 5% level.

** Significant at 1% level.

4 P results were recorded on nine plants only. These results were averaged to ten for statistical analysis.

TABLE 4. EFFECT OF PLANTING DATE ON YIELD
(Average yield per plant, in ounces, weekly intervals)

Week Ending	House 1 Planted Aug 26		House 2 Planted Sept 12	
	Weekly Total	Cumulative Total	Weekly Total	Cumulative Total
Nov. 14	1.5	1.5	.5	.5
Nov. 21	4.0	5.5	4.1	4.6
Nov. 28	9.8	15.3	8.5	13.1
Dec. 5	18.2	33.5	8.0	21.1
Dec. 12	28.5	62.0	9.3	30.4
Dec. 19	25.1	87.1	20.7	51.1
Dec. 26	17.6	104.7	18.1	69.2
Jan. 2	36.3	141.0	35.3	104.5
Jan. 9	13.8	154.8	14.7	119.2
Jan. 16	6.9	161.7	19.0	138.2
Jan. 23	5.5	167.2	15.0	153.2
Jan. 30	6.5	173.7	13.0	166.2
Feb. 6	6.4	180.1	8.5	174.7
Feb. 13	2.7	182.8	6.3	181.0

TABLE 5. SUNSHINE RECORDS AT LINCOLN AND AT KEW

Lincoln (44° S) 1885-1945 Average (hr)			Kew (51° N) 1881-1915 Average (hr)	
	Monthly Total	Daily Average		Daily Average
Apr.	151.2	5.07	Oct	2.94
May	136.1	4.40	Nov	1.73
Jun	115.1	3.84	Dec	1.17
Jul	119.0	3.84	Jan	1.35
Aug	149.2	4.81	Feb	2.10
	Average	4.39	Average	1.85

sufficient to enable the control plants to overcome their disadvantage. Weather records tend to support the validity of this contention.

Treatment was carried out 24 July to 14 August 1958. This period coincided with the normal increase in hours of sunshine, shown in Table 5. The actual daily average in 1958 was 3.83 hours in July, 4.92 in August, and 6.80 in September. The beneficial results of h.p.m.v. shown in England during all the winter months is due to the greatly decreased natural illumination. Hours of sunlight at Kew (Table 5) show that in none of the five winter months is the daily sunshine figure as high as the lowest at Lincoln. It is appreciated that for an accurate comparison, figures for natural illumination excluding



Fig. 1.—Tomato plants after treatment with h.p.m.v. illumination and control plants.

sunlight should also be compared, but none are available for New Zealand.

The tomato plants in the trial were able to show a growth response when sunshine averaged 3.83 hours daily, but this response was lost when sunshine increased to 4.92 hours average and above. For Canterbury it would appear that the border line for response is around four hours of sunshine daily. Plants, for example, raised in late May, illuminated in June and planted early July, are likely to produce a response to illumination. The trial is being repeated in 1959 to test the validity of this contention.

Plant Frames—Pots Trial. The results in Table 2 demonstrate the highly significant increase in early yield, together with overall increase in total yield, which results from raising plants in pots. The larger quantity of soil contained in the pots compared with the plant frames is probably the main factor responsible for this result. Delay in planting and consequently delayed fruiting and demand on soil nutrients would no doubt account for the increase in favour of plant frames for the period 16-31 December.

Potentate—V. 548 Trial. From the commencement of the trial V. 548 was much stronger in growth and rows could be picked out by eye. Cropping commenced at the same time and results in Table 3 show the highly significantly greater yield of V. 548 over Potentate, in the ratio of 129 to 100. The average V. 548 yield of 13 lb 9 oz per plant is very high for plants grown under commercial conditions. These results agree with those of Yen and Newhook (1959). With regard to earliness of V. 548, this aspect should be considered in relation to the overall increase in cropping of this variety compared with Potentate. If the yields per month of Potentate and V. 548 are taken as a

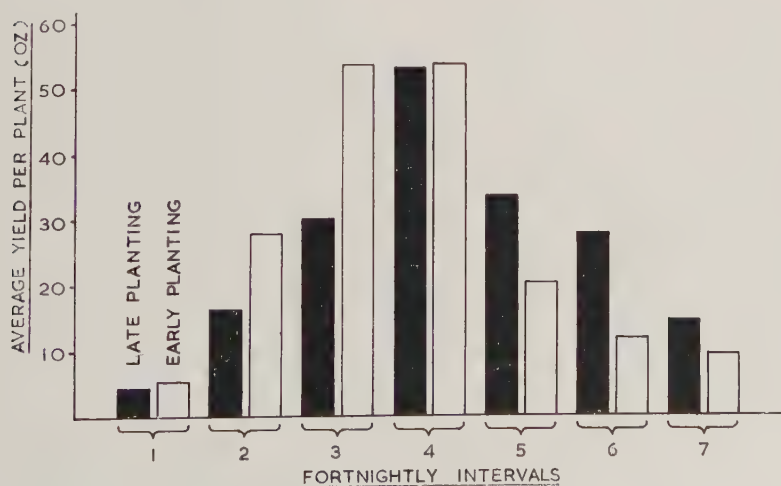


Fig. 2.—Distribution of tomato yield from plants raised together but planted 17 days apart.

percentage of total crop, then it would appear that almost identical cropping trends are followed:

	Nov.	Dec. 1-15	Dec. 16-31	Jan.	Feb.
V. 548	7.4%	13.7%	31.7%	37.2%	9.9%
Potentate	8.7%	15.0%	30.5%	36.2%	9.4%

Delayed Planting. Although not planned as a trial, the delay in planting House 2 gave an opportunity to assess the results of delayed planting upon yield (Table 4). The 17-day delay in planting did not affect total yield, but considerably increased the late yield at the expense of early production. Figure 2 demonstrates this quite clearly and also emphasises that maximum production in both early and late planting occurred in the same fortnight. Financially, however, the effect of the decrease in early yield was quite considerable. Table 6 summarises this effect:

TABLE 6. YIELD AND RETURN FIGURES

Month	Average Price (shllgs/lb)	House 1		House 2	
		Yield/Plant (lb)	Cash Return (shillings)	Yield/Plant (lb)	Cash Return (shillings)
Nov.	2.12	0.93	1.97	0.83	1.75
Dec.	1.28	7.57	10.44	5.60	7.72
Jan.	0.81	2.35	1.90	3.95	3.19
Feb.	0.30	0.58	0.17	0.93	0.27
Total		11.43	14.48	11.31	12.93

The loss per plant in House 2 due to late planting was thus 1.55s., or in the house overall (560 plants) the 17-day delay reduced the cash return by £43 8s. 0d. Extra cost of heating during this period has not been taken into account, but even allowing for this there is still importance in these figures.

ACKNOWLEDGEMENTS

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WEAR IN SHEEP'S TEETH

VI. CHEMICAL COMPOSITION OF TEETH OF
GRAZING SHEEP

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Summary

Excessive wear of incisor teeth of sheep grazing high-fertility (European) pastures occurs in various parts of New Zealand.

Chemical analyses were made of enamels and dentines separated from teeth of 5-year-old ewes bred on farms of different types in various areas. Estimations of calcium, phosphorus, carbonate, sodium, magnesium, nitrogen, citrate, and 19 micro-elements disclosed no differences. There were, however, significant differences in fluorine contents.

Fluorine contents (whole teeth) varied from 28 to 320 p.p.m., the higher amounts being associated with teeth from ewes on pastures top-dressed with phosphatic manures, which contain about 1% fluorine.

The composition of the apatite "bone salt", $(\text{Ca}_3(\text{PO}_4)_2)_n(\text{CaCO}_3)_{1.0}$ varied but slightly, the average values of n for enamel and dentine being 5.2 and 3.5 respectively.

Variations in tooth quality (as judged by relative extent of wear) could not be correlated with differences in chemical composition. The average composition, incidentally, is remarkably similar to that recorded for human teeth.

These results support the earlier theory that substances in the rapidly growing leaves of high-yielding grasses and clovers soften the teeth of sheep grazing them either by dissolving or chelating their inorganic constituents, or by other types of attack with enzymes. These destructive processes would probably be further increased by the abrasive action of fibre in the grasses.

INTRODUCTION

Excessive wear of incisor teeth of sheep occurs in New Zealand on farms in certain areas of improved grassland. In general the defect is most serious on the heavily stocked, lush, low-country farms carrying high-fertility ("European") pasture, predominantly perennial ryegrass (*L. perenne*) and white clover (*T. repens*), and least evident on the lightly stocked hills with their finer, native grasses (Barnicoat 1957).

In Table 1, chemical analyses of different types of pasture and variations in tooth quality are presented in a form modified from part 4 of that paper.

High-production pastures (1), topdressed with phosphatic manures and lime, are richer in protein and in calcium, and are more fibrous, though distinctly lower in polysaccharide content, than the native grasses (3). Weeds and tips of scrub plants (4) from the harder sheep-farming country, are of high protein content, but low in calcium and phosphorus.

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TABLE 1. EXTENT OF WEAR OF TEETH OF 5-YEAR-OLD EWES* IN RELATION TO AVERAGE COMPOSITION OF HERbage EATEN

Herbage Species	Typical 5-year Mouth			Average Chemical Composition (per cent of dry matter)				
	Description	Av. Length Central Incisors 1 ₁₀	Av. Rate of Stocking ewes/acre	Crude Protein	Crude Fibre	Ratio Poly- saccharide† to Crude Protein		Ca/P
						Ca	P	
1. High-production perennial ryegrass and clovers from topdressed pastures	Poor or "gummy"	2.5	5 7	30	16	0.80	0.43	1.9
2. Medium-production grasses from top-dressed fat-lamb "easy" hill country	Poor—fair	3.5	2-4	21	22	0.45	0.33	1.4
3. Native grasses from hill country and other parts used for sheep breeding	Good, excellent, slightly worn	4.5	1 2	9	30	0.29	0.23	1.3
4. Weeds and scrub-tips from "hardest" sheep breeding country	Practically unworn	6	<1	27	18	0.32	0.43	0.9

* Referred to as 5 years old in this article, as in practice, but actually 5½ years. The permanent teeth are cut when sheep are about 1½ years old.

† Polysaccharide = nitrogen-free extract and crude fibre.

The healthiest sheep with the best mouths are bred, as a rule, on high country with herbage of types (3) and (4). If, as generally believed, sheep on lush, low-country pastures ingest 2 to 3 times as much dry matter daily as those on sparse hill country, there would be considerable differences in their dietary intakes. Sheep on improved pastures would ingest more calcium (in particular) and phosphorus, and the intakes of other nutrients, notably protein and polysaccharides, would also differ markedly.

The possible effects of differences in the chemical constituents of herbage on the teeth of grazing sheep is of considerable importance to this particular investigation, and could also be of interest to others, e.g. studies on the effect of diet on the composition and structure of human teeth and their susceptibility to caries, even though sheep's teeth are free from this defect. Previous investigations of this nature have usually been restricted to laboratory experiments with small animals.

Under certain conditions the physical and chemical properties of bone are influenced by the diet (Huggins 1937; Irving 1957). Bone, especially cancellous or spongy bone, such as the inner portions of the vertebrae and mandibles, and the ends of the long bones, are now known to act as the principal "storehouse" of reserve inorganic substances in animals. Such tissues are labile, and changes in composition reflect both the continual demands of the other body tissues, and special demands, such as the early stages of lactation, as well as qualitative and quantitative changes brought about by the deposition of inorganic substances from the diet (Owen 1952; Duckworth and Hill 1953; Duncan 1958).

It was formerly assumed that the chemical composition of the teeth would show similar changes, but it is now known that, while these normally occur in developing teeth, they occur in mature teeth only under extreme conditions of nutritional deficiency, excess, or imbalance.

Apparently Mellanby (1929) was the first to prove experimentally that resistance to dental caries depends on composition and structure of teeth as influenced by dietary factors at the time of tooth development. Sobel (1952) after many years' experimentation has recently reaffirmed this theory.

Sensitivity of developing teeth to dietary influences is much less than that of bone, however (Toverud 1923; Templin and Steenbock 1933; Gaunt and Irving 1939; Gaunt *et al.* 1939; Lund and Armstrong 1942; Smith and Light 1945). Using rats chiefly, these authors have shown that, while the calcium and phosphorus contents of the dry bones were seriously reduced through feeding diets poor in calcium, the teeth were scarcely affected at all.

Sobel and Hanok (1948) fed 23-day-old rats on diets similar except in calcium and phosphorus contents and ratios. After 6 weeks there were considerable differences in tooth composition, but in the carbonate rather than the calcium and phosphorus contents. They state "High calcium low phosphorus diet produces high carbonate, whereas low calcium high phosphorus diet produces low carbonate

tooth (and bone). As carbonate is preferentially dissolved from enamel, one would predict increased caries susceptibility with high carbonate enamel compared to low carbonate enamel." This was later confirmed (Sobel 1952) with cotton rats.

Wynn *et al.* (1956) fed a cariogenic (high sucrose) diet containing ample calcium (0.5%) and varying amounts of phosphorus to 23-day-old rats. In no case was the chemical composition of the enamel and dentine affected, but a progressive reduction in caries resulted as the calcium to phosphorus ratio of the diet changed from 1 to 0.5 to 1 to 1.5. Varying the ratios of calcium to phosphorus in the diet therefore modified the susceptibility of the teeth to caries without perceptibly influencing their chemical composition (or appearance).

Hartles (1951) fed a similar cariogenic diet, containing ample calcium and phosphorus to 21-day-old rats and found that the calcium and phosphorus contents of their enamel and dentine were higher than the controls. He considered that the high carbohydrate intake stimulated the process of mineralisation by maintaining a maximum level of glycogen (for matrix formation) in the developing tooth.

Dietary influences are much more pronounced with developing teeth, such as the continually growing rat incisors, than with mature erupted teeth, but rats' molars behave like mature teeth of other animals, being little subject to dietary influences. This was demonstrated by Armstrong and Barnum (1948) by measuring the turnover of radioactive calcium and phosphorus in the hard tissues of a mature rat (Table 2).

TABLE 2. UPTAKE OF PHOSPHORUS AND CALCIUM BY HARD TISSUES OF A MATURE RAT
(from Armstrong and Barnum 1948)

Tissue	Relative Specific Activity		Ratio Uptake ³² P to ⁴⁵ Ca
	³² P	⁴⁵ Ca	
Femur marrow	143	91	1.57
Femur epiphysis	80	92	0.86
Femur diaphysis	75	82	0.91
Incisor dentine	52	55	0.94
Incisor enamel	27	27	1.00
Molar dentine	14	12	1.16
Molar enamel	2.7	1.2	2.25
Total Body	100.0	100.0	1.00

Rats' molars are relatively stable in comparison with incisors, and the calcified tissues do not exchange calcium and phosphorus in the same proportion, which supports the views of Sobel and Hanok (1948)

and Hartles (1951) that the mineral phases of bone, dentine, and enamel are not identical.

The inorganic component of teeth is apatite, a double salt of calcium phosphate and another calcium salt such as carbonate. About 6% of its calcium may be replaced by sodium, magnesium, and other cations including micro- ("trace") elements. Magnesium, which activates enzymes responsible for transfer and hydrolysis of phosphate groups, and sodium, are present in relatively high concentrations, however.

Lowater and Murray (1937) reported the presence (by spectroscopic methods) of at least 19 elements besides calcium and phosphorus in teeth, but could not relate them to caries incidence. Leicester (1949) summarised papers by several workers showing that deficiencies of micro-elements may seriously impair calcification of bone without necessarily producing specific effects in teeth. Nevertheless, it is to be expected that micro-elements will be of importance to teeth since they are essential constituents of the metalloproteins comprising a number of enzyme systems controlling tissue activities (Lehninger 1950).

Enzymes of oxidation—reduction systems, with which are associated transition elements such as manganese, iron, cobalt, nickel, and possibly vanadium, as well as copper and zinc, are present in teeth and pulp, according to Pincus (1950) and Eichel (1954). Traces of dietary copper (Davies 1950, p. 216), manganese (Smith *et al.* 1944), strontium and vanadium (Rygh 1950), zinc (Follis *et al.* 1941; Nishimura 1953), and molybdenum (Marston 1952), are reported to affect calcification, though the function of molybdenum is obscure, since it may merely be antagonistic to copper (Davies 1950, p. 227). Zinc, barium, and thallium simultaneously are stated to retard the onset of dental caries, while zinc and barium together promote it (Rygh 1950).

It has recently been shown that the lanthanides (rare earths), elements of higher atomic weight, and certain products of atomic fission are "bone seeking" and tend to accumulate in calcified tissues, e.g. the relatively high concentrations of lead and zinc in human teeth (Jenkins 1954).

With the notable exception of fluorine, the essential nature of various micro-elements in the processes or calcification of teeth have not yet been convincingly demonstrated.

That fluorine is of great importance in protecting human dental tissues against caries is now common knowledge. Much work has also been carried out on the effect of fluorine on sheep's teeth, chiefly because of the damaging action of an excess. This defect occurs in various countries, and results usually from stock drinking artesian water containing excessive amounts of fluorine, or consuming licks or feed mixtures containing natural phosphates of high fluorine content (Peirce 1938). This aspect of the work will be discussed further on page 1036.

Anions, including citrate, hydroxyl ion, and fluorine, apparently replace carbonate in the apatite crystals of the tooth structure. Citrate

is universally present in bones and teeth, but, despite intensive research, the nature of its functions is still obscure (Dixon and Perkins 1956).

Nitrogen is present in teeth chiefly as protein, also apparently as a cementing substance. The enamel protein is eu-keratin (Hess *et al.* 1952), whereas the protein of dentine is almost wholly collagen (Hess *et al.* 1953). Protein is also present in dentinal tubules, protoplasmic extensions of the odontoblasts which give the dentine its "living" properties.

TOOTH SAMPLES TAKEN FOR ANALYSES

For this study of sheep's teeth it was considered that the analysis of whole teeth or bones would not be likely to yield unequivocal information concerning the effect of diet. Tedious separations of enamel and dentine were accordingly made, and several methods of chemical analysis were modified to the micro scale because of the small amounts of enamel available.

The sheep's incisor is a hypsodont tooth, i.e. it has a long crown which continues to erupt (emerge) from the gum after cutting, in response to maturity or age, and to wear. Worn teeth may become fully erupted and abraded to gum level at an early stage, whereas teeth from older animals on country causing little wear may remain long and unworn ("chisel edged") with a considerable portion of the crown unerupted, i.e. below gum level (Barnicoat, part 3, 1957).

The sheep's incisor, both structurally and functionally, resembles the human incisor rather than the tusk-like, continually growing front tooth of the rat. It would therefore be expected that differences in composition between teeth of sheep from various sources would be most influenced by nutritional factors during development rather than later. For this reason, care was taken to ensure that teeth analysed came only from sheep born and bred on the particular farms selected for this investigation. The samples analysed were permanent incisors of "full mouthed" ($3\frac{1}{2}$ years and older) Romney breeding ewes from long-established flocks on farms in the North Island of New Zealand, chiefly the Manawatu and Taihape areas.

The soils of these farms represented a fair range* of the mildly acid types found in sheep-farming areas, and some had been rather heavily topdressed, chiefly with superphosphate and lime. Since the farms had been stocked with sheep during the previous 30 to 60 years, any compositional peculiarities of the herbage, including those of a cumulative nature, should have become well established.

In the North Island of New Zealand, sheep feed consists principally of grasses and clovers, and usually hay and crops produced on the farms, but these supplementary feedstuffs amount to only a minor proportion of the whole. (When these tooth samples were collected, concentrates were little used except on stud farms, and were not fed to breeding ewes.)

* Soil types, according to N.Z. Soil Bureau's (*Bull. n.s.* 5) Classification were:—13, 18gH, 20e, 25cH, 29H, 65cH, 113b, 114a, 114b, 116, 116b, 117a, 118a, 122, and 124.

Because of the "natural" state of the food, and lack of complications from differences in breeding, and because of the fact that the ewes and their ancestors had been bred on the same farm, usually for a number of generations, many variables encountered, for example, with human experiments of this nature were avoided.

METHODS

Preparation of Samples

Jaws were usually preserved by drying in the sun and were often kept for several months pending analysis. Teeth were extracted by softening the surrounding tissues with boiling water, were scraped to remove congealed blood, tissue, grass stains, and dirt, and were then dried at room temperature. After grinding to 100 mesh in percussion and mechanical mortars, the powders were soaked overnight in alcohol and extracted in a soxhlet with ether for 6 hours. Part of the air-dry powder was used for fluorine analysis, and the remainder was separated into enamel and dentine by the flotation method.

Separation of Enamel and Dentine: Two methods were employed:

- (i) Flotation method (Manly and Hodge 1939) for samples analysed for organic constituents and carbonate, and for most enamel samples because their purity could be checked as exceeding 99% by Becke's line test, and
- (ii) Dissection of enamel and dentine (the simpler method) by prising them apart after destroying organic matter by heating for 2 or 3 hours at about 400°C. Micro-elements in the enzymes of the pulp and odontoblasts were thereby conserved in samples used for spectroscopic analysis.

Chemical Methods of Analysis

Since micro methods were employed in most cases, especially for enamels, estimations were usually made in triplicate. The methods used were the following:

Moisture: 6 hours at 105°C.

Calcium: Oxalate precipitation at pH 3 (Washburn and Shear 1932), and permanganate titration.

Phosphorus: Gravimetric ("Lorenz") method. (Belcher and Wilson 1955).

Citrate: Adapted from McCardle's (1955) modification of the methods of Weil-Malherbe and Bone (1949) and Taylor (1953).

Nitrogen: Micro-Nessler (Umbreit *et al.* 1957); with distillation of ammonia in Markham (1942) still in order to avoid clouding of Nessler solution by calcium salts.

Carbonate: Conway (1957) No. 2 vessel, placing the tooth powder in the central compartment.

Sodium and Magnesium: Atomic absorption spectrophotometric method of Allan (1958).

TABLE 3. CHEMICAL COMPOSITION OF SHEEP'S TEETH
Actual range of values shown in parentheses, number of samples in square brackets. Results in parts per cent dry basis, except fluorine (p.p.m.).

Constituent	Enamel		Dentine			
	Averages	Teeth with Least Wear	Teeth with Most Wear	Average	Teeth with Least Wear	Teeth with Most Wear
Calcium*	37.6 (37.1-38.2) [20]	37.6 [6]	37.5 [6]	35.05 (34.3-35.6) [10]	35.0 [4]	35.2 [4]
Phosphorus*	18.8 (18.2-19.2) [20]	18.6 [6]	19.0 [6]	18.0 (17.9-18.4) [10]	18.25 [4]	17.9 [4]
Ratio Calcium/ Phosphorus*	2.0 (1.93-2.06) [20]	2.02 [4]	1.97 [4]	1.95 (1.90-1.98) [10]	1.92 [4]	1.96 [4]
Magnesium*	0.28 (0.24-0.30) [20]	0.28 [8]	0.28 [8]	1.08 (1.02-1.16) [5]	—	—
Sodium*	0.55 (0.51-0.68) [20]	0.57 [8]	0.54 [8]	0.71 (0.68-0.74) [5]	—	—
Carbonate** (as CO ₂)	2.53 (2.30-2.89) [25]	2.52 [6]	2.54 [7]	2.79 (2.45-3.14) [25]	2.80 [6]	2.79 [7]
Citrate** (as citric acid)	0.057 (0.044-0.077) [25]	0.061 [5]	0.057 [5]	0.76 (0.68-0.83) [20]	0.74 [5]	0.76 [5]
Nitrogen***	0.04 [22]	0.04 [5]	0.06 [5]	3.34 (2.85-3.62) [22]	3.29 [5]	3.51 [5]
Fluorine*			****	149 (43-290) [20]	157 (43-280) [8]	155 (62-290) [7]

* Ash.

** Fat-free dry matter, separated by flotation method (sp. gr. 2.70).

*** Similar treatment (sp. gr. 2.85).

**** Ground mixed whole teeth, mainly dentine (p.p.m.)

TABLE 3.—Continued—CHEMICAL COMPOSITION OF SHEEP'S TEETH
Elements estimated spectroscopically (results in parts per million of ash)

Constituent	Enamel			Dentine		
	Average	Teeth with Least Wear	Teeth with Most Wear	Average	Teeth with Least Wear	Teeth with Most Wear
Aluminium	[6] 50	[3] 45	[3] 55	[6] 45	[3] 40	[3] 50
Silicon	—	—	—	110	70	150
Titanium	<10	<10	<10	25	10	40
Vanadium	—	—	—	< 1	< 1	< 1
Chromium	—	—	—	1.5	1	2
Manganese	<10	<10	<10	< 10	< 10	< 10
Iron	30	30	30	115	140	90
Cobalt	—	—	—	< 1	< 1	< 1
Nickel	—	—	—	1.5	1	2
Copper	45	40	60	4	< 3	5
Zinc	—	—	—	<100	<100	<100
Gallium	—	—	—	< 1	< 1	< 1
Germanium	—	—	—	< 3	< 3	< 3
Strontium	620	630	600	870	870	870
Molybdenum	1.5	1	2	< 0.1	< 0.1	< 0.1
Silver	—	—	—	< 0.1	< 0.1	< 0.1
Tin	—	—	—	< 2	< 2	< 2
Barium	330	390	280	870	870	870
Lead	—	—	—	3	3	3

Fluorine: Ashing whole ground teeth with magnesium acetate, below 570°C (Crutchfield 1942); distilling with perchloric acid (Anon. 1944); and titration using Solochrome Blue as indicator (Medical Research Council 1949).

Micro-elements: Spectroscopic carbon-arc emission method (Wells 1956).

RESULTS

In Table 3 are recorded analyses of enamels and dentines of sheep's teeth. In addition to the averages, values for teeth with least and most wear (i.e. typical of animals in Table 1, "Herbage Groups" 4 and 3, and 2 and 1 respectively) are also reported. The results of the analyses are comparable, since they refer to similar teeth of full-mouthed ewes, chiefly 5-year-olds.

The descriptions "teeth with least wear" and "teeth with most wear" are preferred to "best teeth" and "poorest teeth" since the two

TABLE 4. STOICHIOMETRIC RELATIONSHIPS IN THE ENAMELS AND DENTINES OF SHEEP'S TEETH
(Dry, protein-free basis)

- 1 Representative sample from 20 full-mouthed sheep.
- 2 Central incisors, recently erupted, from 18-month-old ewe.
- 3 Teeth from 5-year-old sheep on a farm noted for outstandingly good mouths.
- 4 Teeth (5-year-ewes) with highest CO₂ content.
- 5 Teeth (5-year-ewes) with lowest CO₂ content

Member	Enamel					Dentine				
	1	2	3	4	5	1	2	3	4	5
Calcium %	37.6	37.7	37.2	37.6	37.6	35.05	34.3	35.6	35.05	35.05
Phosphorus %	18.8	19.0	18.15	18.8	18.8	18.0	17.65	18.15	18.0	18.0
Carbon dioxide %	2.57	2.42	2.40	2.94	2.34	3.67	3.65	3.65	3.83	3.31
Calcium (mm%) × 100	93.8	94.0	92.8	93.8	93.8	87.4	85.6	88.8	87.4	87.4
Phosphorus (mm%) × 100	60.6	61.2	58.5	60.6	60.6	58.0	56.9	58.5	58.0	58.0
Carbonate (CO ₂) (mm%) × 100	5.84	5.50	5.45	6.68	5.32	8.34	8.29	8.29	8.70	7.52
Ca : PO ₄ ratio*	1.55	1.54	1.59	1.55	1.55	1.51	1.50	1.52	1.51	1.51
PO ₄ : 2CO ₃ ratio**	5.2	5.6	5.4	4.5	5.7	3.5	3.4	3.5	3.3	3.9

** The PO₄ : 2CO₃ ratio = n in the formula for apatite, (Ca₁₀(PO₄)₂)_n(CaCO₃)₁₀ when the molecular ratio* Ca:PO₄ is 1.50. This is equivalent to a weight ratio Ca/P of 1.94.

sets of terms are not necessarily synonymous from the viewpoint of practical farming.

DISCUSSION

With the exception of the fluorine, and possibly silicon, contents, results in Table 3 are remarkably uniform, and lend no support to the theory that differences in the chemical composition of enamel and dentine of sheep's teeth influence their structure or physical properties and consequently the rates at which they will tend to wear. Nor do they accord with the theory that the soil type, or herbage species influence their chemical composition, or physical properties.

The soils and pastures on the farms from which the jaws came are fairly representative of those on the majority of sheep farms in the North Island of New Zealand. Indeed, the results strengthen the theory discussed in part 4 of this series (Barnicoat 1957), that the erosive (chemical or biochemical action) and abrasive actions of the herbage, determine in the main the rate of wear of teeth of grazing sheep.

Calcium, Phosphorus, and Carbonate. The remarkable uniformity in composition of sheep's teeth is further illustrated by the consistent evenness in Ca/P ratios, but the stoichiometric ratios (Sobel and Hanok 1948) give an even better understanding of the chemical composition than weight ratios (Table 4).

In Table 4 it is apparent that both enamels and dentines of widely different types of teeth have similar stoichiometric ratios of Ca, PO_4 , and CO_3 . Sobel (1953) considers the normal range of n in "bone salts" to be 2.1–9.3, in some species dentine being the higher. The average values [columns 1] for sheep's enamel and dentine are 5.2 and 3.5 respectively.

Magnesium and Sodium. The compositional range is small and unrelated to other characteristics.

Organic Constituents. (i) *Nitrogen.* Nitrogen contents of dentine are fairly uniform, being equivalent to about 21% protein on the dry basis. In enamel the estimate of nitrogen tends to be high, according to Stack (1954), through contamination with material rich in protein from the enamel-dentine junction. Since the enamels separated by flotation of dentine at sp. gr. 2.70 were of only about 99% purity, some were re-separated with bromoform of sp. gr. 2.85, the results being:

Re-separated at sp. gr. 2.85	Enamel separated at sp. gr. 2.70			= 0.17% nitrogen
	{ 83% sinking			= 0.04% nitrogen
	{ 17% floating			= 0.70% nitrogen
	original composition calculated from re-separated fractions			= 0.15% nitrogen

The lowest value for nitrogen in the re-separated material is presumably that of the least-contaminated enamel, and is returned as such, i.e. 0.04% N equivalent to about 0.25% protein.

(ii) *Citrate.* Differences in concentration in enamel and dentine are not related to tooth quality. Citrate/N ratios in enamel and dentine are approximately 1.5 and 4.5 respectively.

Fluorine. Fluorine is the only constituent showing wide variations. In 50 samples of mixed (whole) teeth from 21 farms, fluorine contents ranged from 28 to 320 p.p.m. averaging 149 p.p.m. It might be expected that teeth of low fluorine content would be the most rapidly worn, since human teeth of this type are susceptible to caries; or that teeth of high fluorine content might appear bleached or mottled, with irregularities in shape, typical of fluorosed sheep's teeth in Australia (Peirce 1938), although fluorine contents never approached the values even of the most mildly affected Australian samples.

Such defects were not observed however, nor were there any indications that the proportion of fluorine in New Zealand sheep's teeth influences their tendency to wear. If there is any such relationship, it is obscured by other factors.

Since other constituents vary so little, the exceptionally wide variations in fluorine may seem surprising. They were apparent even in the categories resulting from classifying teeth according to their amount of wear.

The fluorine content of soil shows little variation (Gemmell 1945). It would appear that fluorine in sheep's teeth is derived from that applied in artificial manure, since both superphosphate and basic slag contain about 1% fluorine. For example, teeth from an experimental area at Massey College, consistently and fairly heavily topdressed for 14 years, contained on the average 290 p.p.m. fluorine, whereas teeth from a neighbouring farm on the same type of soil, little topdressed, contained only 123 p.p.m.

The lowest values appear to be associated with farms which have not been artificially manured. These values are low in comparison with those reported overseas, but some of the best teeth came from these properties.

Other Micro-elements. Considerable variations occurred in the concentrations of certain micro-elements in six samples of teeth of widely different types selected for this study.

Titanium contents of dentine differed merely because of the relatively high concentration in one sample. Iron in dentine is presumably derived from blood retained in the pulp, but the high concentration of copper in the enamel, in comparison with that in the dentine, is interesting and cannot be explained. Strontium and barium are congeners of calcium, and their variations are not regarded as unusual. Differences in the silicon contents of dentine are sufficiently interesting to warrant further study, though they may merely result from contamination with soil or herbage since some grasses contain considerable amounts of silica.

Titanium in enamel, and manganese, zinc, and cobalt contents, were below limits of detection in the small samples available.

CHEMICAL COMPOSITION OF TEETH IN RELATION TO AGE

Sheep's teeth develop below the gum 4 months or longer before erupting and calcification does not appear to be complete until they

are actually in use. The eight incisors of the lower jaw are cut in pairs at four different stages. In $5\frac{1}{2}$ -year-old ewes the central pair, the earliest to appear (i.e. at the "two tooth" stage), will have been about three times as long in service as the latest to erupt, the two "corner teeth" (which are strictly canines).

It might be expected that the chemical composition of teeth in an individual jaw would vary according to age. That this is not so is apparent from analyses in Table 5.

TABLE 5. VARIATIONS IN CHEMICAL COMPOSITION BETWEEN TEETH IN INDIVIDUAL JAWS OF $5\frac{1}{2}$ -YEAR-OLD SHEEP

Description of Teeth	Central Incisors	Second Incisors	Third Incisors	"Corner Teeth" (strictly canines)
"Saleyards" Description of Mouth	2-tooth	4-tooth	6-tooth	Full Mouth
Approximate Age of Sheep	16	24	32	48 months
Approximate Length of Time in Service of Teeth in $5\frac{1}{2}$ -year-old Ewe's Mouth	50	42	34	18 months
Analyses	%	%	%	%
Citrate in enamel: "fair" mouth	0.057	0.048	0.048	0.041
unworn ("good") mouth	0.055	0.043	0.053	0.043
Nitrogen in dentine: "fair" mouth	3.4	—	3.4	3.7
unworn ("good") mouth	3.3	3.4	3.2	3.3
Carbonate (as CO_2) in enamel: "fair" mouth	2.68	2.89	2.28	2.57
unworn ("good") mouth	2.70	2.60	2.42	2.78
in dentine: "fair" mouth	2.94	—	2.82	3.11
unworn ("good") mouth	3.14	2.82	2.82	2.94
	p.p.m.	p.p.m.	p.p.m.	p.p.m.
Fluorine in whole teeth: little worn ("good") mouths	44 60 130	57 54 121	38 50 105	31 60 125
well worn ("poor") mouths	116 186 111	99 210 122	166 202 125	93 205 133
Average for fluorine	108	111	114	110

It will be seen that results for different teeth in each mouth are similar, indicating that analyses of the central incisors would be representative of the whole set. It is usually in the developing stage that their composition is most susceptible to factors influencing composition, though some workers (e.g. Perry and Armstrong 1941) consider that fluorine is taken up readily even by mature, erupted teeth. Apparently chemical analyses of the central incisors of younger sheep would be quite suitable for purposes of comparison. The central incisors are good sources of material since they contain almost as much tooth substance as the other 6 teeth.

SIMILARITY IN COMPOSITION BETWEEN SHEEP'S INCISORS AND THOSE OF OTHER SPECIES

Results of chemical analysis of sheep's teeth are not only remarkably even, but also strikingly similar to those recorded for other species, particularly man (Leicester 1949; Jenkins 1954); as shown in Table 6).

TABLE 6. AVERAGE COMPOSITION OF SHEEP'S AND HUMAN TEETH

	Enamel		Dentine	
	Sheep %	Human %	Sheep %	Human %
Calcium (ash)	37.6	38	35.1	38
Phosphorus (ash)	18.8	18	18.0	18
Ca/P (ash)	2.00	2.1	1.95	2.1
Sodium (ash)	0.55	0.7	0.71	0.3
Magnesium (ash)	0.28	0.4	1.08	1.2
Carbonate (as CO ₂) fat-free dry tooth	2.53	2.5	2.79	3.25
Citrate, fat-free dry tooth	0.057	0.1	0.76	0.9
Nitrogen, fat-free dry tooth	0.04	0.06	3.34	3.54
Fluorine, fat-free dry-tooth	—	—	0.0159*	0.0163†

* Whole teeth.

† Dentine (Armstrong and Brekhus 1938).

With the single exception of fluorine, attempts by numerous workers to relate the incidence of caries in human teeth to chemical composition have not proved encouraging. In the simpler more closely defined investigation reported in this paper concerning excessive rates of wear of incisor teeth of sheep grazing high-fertility pastures, still no differences in chemical composition could be established other than fluorine which, however, did not appear to influence tooth quality.

Increased rates of wear of teeth of sheep grazing high-fertility pastures are probably caused by substances in their rapidly growing leaves attacking the teeth. These substances apparently soften the

teeth, causing chemical changes in the inorganic constituents (solution or chelation), or other changes of enzymic origin, either of which could be increased through abrasion caused by fibre in the grasses.

Causative factors of a similar nature are also of interest to workers in the field of human dental caries research. While caries is never found in incisor teeth of sheep in New Zealand, it is interesting to speculate that the fundamental causes underlying human dental caries and wear in sheep's teeth may be similar in nature.

The problem of wear in sheep's teeth is still under investigation.

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PHOTOSENSITIVITY DISEASES IN NEW ZEALAND

XVII. THE ASSOCIATION OF *Sporidesmium bakeri* WITH FACIAL ECZEMA

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Summary

Mycelium from a fungus subsequently identified as *Sporidesmium bakeri* Syd., a species not previously detected in New Zealand, was observed on pasture. Facial eczema occurred in lambs grazing this pasture and in guinea pigs fed dried grass mown from the area.

A spore sample collected from the same area comprised predominantly *S. bakeri* and gave a positive beaker test.

Cultures of this fungus when fed to guinea pigs and lambs produced characteristic facial-eczema liver lesions as well as icterus and photosensitivity in the latter species. The toxin was detected in cultures which varied in incubation time and media. Mycelium as well as conidia were toxic.

INTRODUCTION

Facial eczema was first reported by Gilruth in 1908. Filmer has reviewed progress in research since then (Filmer 1958). The disease was confirmed by Filmer as one involving obliterative cholangitis produced by a hepatotoxin, dietetic in origin (Simpson *et al.* 1957). Following the most serious outbreak of the disease in 1938, intensive efforts failed to associate any pasture micro-organism with the disease (Neill 1940; Cunningham 1958). In consequence the alternative theory that a plant toxin was responsible dominated subsequent research.

In 1945 Manutuke Research Station was established. One of its main objectives was to collect and preserve large quantities of toxic grass to enable chemists to isolate the liver-damaging factor. For a long time progress was limited by lack of a test animal smaller than the sheep. However in 1952 Evans and co-workers found that the guinea pig could be used to test for the presence of the toxin in samples of dried grass (Evans *et al.* 1957).

Perrin (1957) developed a bio-assay technique with the guinea pig and this enabled White (1958a) to prepare concentrates of the hepatotoxin from grass. The toxin was very unstable and present in low concentration even in highly toxic samples of dried grass. Many anomalies in the occurrence of facial eczema suggested that the toxin was not merely a product of rapid pasture growth, but that some hitherto undetermined factor might influence the development of toxicity (Clare 1955).

In 1956 Perrin found that toxic grass samples were as a rule those which gave a positive beaker test (Perrin 1959). This suggested that discovery of the origin of the substance responsible for the beaker test

might lead also to the origin of the toxin. Chemical isolation of the beaker test substance in an impure state showed that it resembled products isolated from some micro-organisms (White 1958b).

In February 1957 staff of the Biology Section of the Soil Bureau, Department of Scientific and Industrial Research, who were already conducting a broad investigation of forest and pasture soil microbiology, met those working at Ruakura on facial eczema, to discuss a further microbiological approach to this problem. This paper reports the steps leading to the discovery that the liver-damaging factor of the disease originated from the fungus *Sporidesmium bakeri* Syd. (Percival and Thornton 1958; Thornton and Percival 1959; Anon. 1958).

EXPERIMENTAL PROCEDURES 1957

Field. As lack of highly toxic grass was still retarding progress in the chemical purification of the toxin, an extended grass-collection programme commenced in 1957. In addition to the Manutuke Research Station farm, grass was collected from four other farms in the Gisborne district when eczema was common, and from eleven farms in the Waikato district. On each of these properties two-acre areas were selected, and every second day sufficient grass was mown to provide a 40-lb sample of dried material. Grass collection began at the onset of autumn rains and continued until the end of April. The dried pasture samples were tested for the presence of the toxin by the guinea pig assay technique. These procedures were also expected to provide more accurate data on the duration and levels of toxicity of pasture than had been possible by the lamb grazing technique as described by Simpson *et al.* (1957). Such data would also be useful to check the accuracy of the beaker test/toxicity relationship and to examine any possible association of pasture micro-organism with facial eczema.

Microbiological. Commencing in February 1957, soil and pasture samples were obtained at intervals throughout the year from one of the worst eczema areas, the polo field at Claudelands, Hamilton. During periods when eczema was expected to occur, sampling was intensified, not only at Claudelands, but also on the other suspect areas. The staff of the Soil Bureau identified yeasts, fungi, and bacteria present, and followed their seasonal population changes.

EXPERIMENTAL RESULTS 1957

Gisborne District. Dried-grass samples obtained from four of the five areas in the Gisborne district proved toxic when fed to guinea pigs. The beaker-test results showed that the test could be used with a high degree of accuracy to indicate the onset of toxicity (Clare *et al.* 1959). Although positive beaker tests were obtained after toxicity had disappeared, it was rare for the toxin to be found in samples showing a negative beaker test.

Waikato District. Only three of the 440 grass samples serially collected over the eleven different Waikato areas proved toxic when tested with guinea pigs. One of these toxic samples was obtained from the two-acre experimental area at Claudelands and the other two from a farm near Matamata.

Microbiological. Di Menna (1959) and Thornton and Ross (1959) have described the isolation and cultivation of yeasts and fungi respectively from the samples collected. In October 1957 Di Menna and Thornton (pers. comm.) reported that guinea pigs fed cultures of the predominant species of yeasts, fungi, and bacteria isolated from the soil and grass samples collected during 1957 showed no macroscopic symptoms of eczema liver damage. However, since the predominant species of micro-organisms had been tested for the presence of the eczema toxin under only a narrow range of cultural conditions, it was impossible to be certain whether absence of toxicity was due to failure to isolate the micro-organism responsible, or to failure to provide suitable cultural conditions for the production of the toxin.

Beaker-test Application. Microbiological samples were subjected to the beaker test in an attempt to locate the source of this substance. The predominant summer species of fungi, yeasts, and bacteria were grown at the Soil Bureau under various cultural conditions from December 1957 to May 1958 and forwarded to Ruakura for beaker testing; all cultures proved negative.

EXPERIMENTAL PROCEDURES 1958

Gisborne District. Owing to unfavourable weather conditions, no facial eczema occurred in the Gisborne area and all beaker-tested grass samples were negative.

Waikato District. Large quantities of grass were collected, but by no means all of it gave a positive beaker test. In many cases when a large quantity of grass was cut on the day after a small sample of grass had given a positive beaker test, the larger sample of material gave a negative test. The polo field at Claudelands was not available for routine sampling for the entire eczema season (January to April inclusive), and samples were taken from an adjoining field. A beaker-test check was kept on the polo field at weekly intervals, and a sample cut on 13 March gave the highest-grade beaker test (grade 6) for the season to date. This high beaker-test grade was confirmed the following day. Large quantities of grass were, therefore, cut and dried in expectation of the material being toxic. Each day from 13-20 March inclusive grass samples were obtained from different areas of the Claudelands polo field and tested. From the area which gave the highest beaker-test grading large quantities of grass were mown and dried. Because the beaker-test values of samples collected on the same day from different areas of the polo field varied widely, sampling procedures were investigated. Results show that:

- (a) Grass mown at low levels invariably gave higher beaker-test gradings than high-level cuts.
- (b) Dead-grass patches produced high beaker-test gradings.
- (c) Soil and roots to 1 in. depth gave negative beaker tests.
- (d) Positive grass samples when washed with water and dried retained their beaker-test grading.

- (e) The regrowth from areas that had been mown a few days previously sometimes continued to give positive beaker tests.

It was concluded that high beaker-test gradings were primarily associated with grass samples containing a high proportion of dead grass.

FUNGAL ORIGIN OF BEAKER-TEST SUBSTANCE

On 15 March 1958, during the course of the above investigations, fungal mycelium was observed on and about dead leaves at the base of grass plants, and four turf samples were forwarded to the Soil Bureau for identification and examination.

On 23 March the operator of the gang mower noticed a black dust hovering over the mower as the area was being prepared for polo. A light dusting of this suspected spore material was collected from the mower and subjected to the beaker test. It produced a strong positive reaction (grade 6).

Further samples of less-pure spore material scraped from the lawn-mower also produced strongly positive beaker tests. In one of these samples the substance responsible for the beaker-test reaction was present at a level of 1 : 5000. Although this substance had previously been obtained from toxic grass it had never been found present at a concentration higher than 1 : 50,000 (White 1958a). A sample of the suspected spore material was sent to the Soil Bureau for identification. In April the predominant spores were tentatively identified as *Stemphylium botryosum*, a fungal species previously reported by Dye and Vernon (1952) as being very common and widespread throughout New Zealand. In May it was reported (R. H. Thornton pers. comm.) that each of the four mycelial samples collected on 15 March contained the same dominant species of fungi. It was notable that this fungus should prove to be the same species as the spores.

In October the Commonwealth Mycological Institute at Kew identified the fungus as *Sporidesmium bakeri* Syd., a fungus with a more restricted geographical distribution than *Stemphylium*. This was more compatible with current knowledge of the incidence and distribution of facial eczema in the field. Following the discovery of the spores, grass samples collected from the Claudelands area during the investigation into the effect of sampling methods upon beaker-test levels were examined microscopically. *S. bakeri* spores were present on both green and dead leaves, but appeared more prevalent on the dead material which had previously shown higher beaker-test grades. It appeared, therefore, that the beaker-test substance was derived from the conidia of the fungus *S. bakeri*.

To obtain a sporulating culture to test this hypothesis Thornton and Ross (1959) ultimately selected from strain CL (derived from the Claudelands mycelial samples), a high-sporing strain, Strain C, which was incubated for eleven days on a potato-carrot-extract agar medium at 24°C. Spores scraped from this culture and others similarly grown at Ruakura Animal Research Station all gave positive results when tested at Ruakura. A relatively pure sample of spores was then obtained

by washing a culture with water. On analysis this showed the presence of the major component of the beaker-test material, the substance with melting point 260°C. The conidia of *S. bakeri* were thus shown to be a source of the beaker-test substance.

Presence of Fungus and the Occurrence of Facial Eczema

Waikato District. From the commencement of autumn rains on 18 February, five lambs were grazed on the Claudelands area. When slaughtered on 16 April, macroscopic examination of their livers revealed one with facial-eczema lesions classified as severe, one with a very mildly damaged liver and three with normal livers. It was apparent that this area had been toxic at some time between 18 February and 16 April.

Samples from the large quantities of grass that were mown and dried daily from 13-20 March from the Claudelands polo field were tested for toxicity by feeding to guinea pigs for the standard 35-day test period (Perrin 1957). The testing of each sample with three guinea pigs started within a week of the collection date. Feed samples were also tested and the results of these tests are shown in Table 1.

TABLE 1. TESTS ON CLAUDELANDS GRASS SAMPLE

Date Collected	Location	Liver Damage	Beaker Test Grade
March 13	East	Very severe	6
14	East	Severe	5
15	South	Very mild	5
16	South	Mod. severe	6
17	North	Moderate	6
18	Centre	Mild to Mod.	6
19	East (regrowth)	Mild	4
20	North	Very mild	3

The data in Table 1 indicate that the grass was toxic during the period 13-20 March, and that samples from the different areas showed considerable daily variation in toxicity. Also, the turfs containing the mycelial samples of 15 March were collected at a time when the grass was toxic. Whereas the beaker-test grade varied only between 5 and 6 for samples collected between 13-18 March inclusive, the toxicity levels of these samples caused liver damage in guinea pigs which varied from very mild to severe. An attempt was made to assess the toxicity levels of these grass samples by the incidence of spores. This was not successful except in the case of the sample collected on 20 March which contained comparatively few spores, was only very mildly toxic, and provided the lowest beaker-test grading of the series.

THE TESTING OF *Sporidesmium bakeri* FOR TOXICITY

The demonstration that the spores of *S. bakeri* contained the beaker-test substance and the relationship between beaker test and toxicity strongly suggested a relationship between fungus and facial eczema toxin. In addition, the fact that this fungal species had not

been detected in soil and pasture samples previously examined, and the association of both mycelium and spores of this fungus with toxic grass, emphasised the need to test *S. bakeri* for possible production of the facial-eczema toxin. Many factors such as strain of fungus, type of medium, incubation period, and incubation temperature were also recognised as being capable of affecting toxin production.

Testing Methods

In the first attempts to test *S. bakeri* for toxicity with the high-sporing Strain C, it was incubated under diffused natural light conditions at 24°C on potato-carrot (200 g each per litre) extract agar medium in Roux flasks (200 g medium) for incubation periods of 4, 7, 11, 14, and 21 days.

Cultures from each incubation period were separately tested as cultures (spore-bearing mycelium plus medium) and as extracts of cultures. The toxicity tests were based on the techniques developed by Perrin (1957). In the feeding of cultures, macerated spore-bearing mycelium and media were offered daily for 35 days at a level of 25 g mixed with 20 g of dried ground non-toxic grass. Cultures were fed for a longer period and to larger guinea pigs than were extracts, in order to ensure a reasonable intake of such an unusual diet.

To allow for unpalatable cultures, low toxicity, and loss of toxicity in the preparation of the extracts, the extracts of cultures were fed at approximately three times the level of the cultures. These extracts were prepared as follows: Macerated cultures from nine Roux flasks (approx. 1700 g) were extracted with three successive 250-ml volumes of ether. The ether extracts were combined and evaporated under an air blast. The watery residue was re-extracted three times with minimum amounts of ether and the combined ether extracts were evaporated and dried under vacuum. The extracts were then dissolved in a small volume of ether and dispersed on to 168 g of dried ground non-toxic grass. Extracts were fed to guinea pigs for 21 days, the daily ration comprising 8 g of the treated grass, mixed with 4 g whole milk powder and 8 ml water. Each day's ration thus contained the extract from approximately 80 g of fungal culture. Feeding was continued for a further 7 days with 10 g non-toxic grass, 5 g milk powder, and 10 ml water so that the complete test period was 28 days.

All experimental rations were stored in a refrigerator at 0–5°C until fed. Food consumption was measured daily and the guinea pigs were weighed twice weekly. Drinking water was provided *ad lib*. At the end of the experimental period the animals were killed, their livers were weighed and the level of toxicity was assessed by the degree of macroscopic liver damage in the manner described by Perrin (1957). Liver slices were fixed in formal saline and sections examined histologically.

One of the effects of the facial eczema toxin on the guinea pig liver is to increase its size. In this study hepatomegaly was considered to have occurred when the liver weight exceeded by 2 g the normal weight of the liver as estimated by the equation $Y = 1.4 + 0.33X$ (Perrin 1957) where Y = normal liver weight and X = the maximum body weight attained.

Test of Fungal Cultures

Results of feeding fungal cultures comprising spore-bearing mycelium and medium are presented in Table 2. The data include those for two control pigs which were offered non-toxic grass and uninoculated media at the same daily level as the pigs fed the experimental rations. Because of contamination, no 21-day-incubated cultures were tested.

TABLE 2. TESTS OF CULTURES

Pig No.	Incub. Time (days)	Culture Intake		Survival Time (days)	Body Weights (g)			Liver Wt. (g)	Liver Damage	
		Total (g)	Av./Day (g)		Init.	Max.	Final		(mac.)	(hist.)
1	Control	867	25	35	172	314	314	9.8	Nil.	—
2	Control	846	24	35	163	266	266	9.5	Nil.	—
4	4	335	21	16	171	185	156	10.7*	Sev.	+
7	7	689	20	35	175	235	225	25.2*	Sev.	+
11	11	475	21	23	183	205	205	20.0*	Sev.	+
14	14	705	20	35	156	231	208	20.6*	Sev.	+

* Liver heavier than normal.

The two pigs fed the control ration had high intakes with daily averages of 25 and 24 g respectively, which in the case of pig No. 1 represented the whole of the media portion of the ration. Both animals made normal growth throughout the 35-day test period with weight gains of 142 g and 103 g. On post-mortem they had normal livers. Each of the four pigs fed the rations containing the *S. bakeri* cultures ate less than the control pigs. Although two pigs, Nos. 4 and 11, survived for only 16 days and 23 days respectively, nevertheless their average daily intakes of 21 g were comparable with the 20 g intakes of pigs Nos. 7 and 14, both of which completed the five-week test period. The intakes of these two latter pigs fell most during their fourth and fifth experimental weeks, whereas intakes of pigs Nos. 4 and 11 dropped markedly two days before death. The cultures appeared to have been reasonably palatable. All four animals developed macroscopic liver lesions which were confirmed histologically as being indistinguishable from the liver lesions of guinea pigs fed toxic grass. All livers were uniformly pale and enlarged and were graded as severely damaged. The livers of the pigs fed the cultures incubated for 7, 11, and 14 days were grossly enlarged and rubbery in texture. The liver of pig No. 11 showed necrotic areas. The short survival time of 16 days for pig No. 4 could account for its comparatively light liver (Perrin 1957). The growth of all pigs on the experimental diets was retarded, and no pig attained the consistent 20 to 25 g weekly weight gain exhibited by the animals on the control diets. Pigs Nos. 4 and 11 attained maximum body weight after making poor weight gains of 14 g over 10 days and 22 g over 16 days respectively. Thereafter pig No. 14 lost weight while

the weight of No. 11 remained unchanged. Maximum weight was attained after gains of 60 g in 25 days and 75 g in 29 days respectively for pig Nos. 7 and 14. Thereafter they both lost weight. Conidia of *S. bakeri* were very numerous in the 4-day and older cultures. Severe eczema liver damage was produced with only 335 g of fungal culture.

Tests of Ether Extract of Fungal Cultures

Results obtained from feeding extracts of fungal cultures for the several incubation periods are summarised in Table 3.

TABLE 3. TESTS OF EXTRACTS

Pig No.	Incub. Time (days)	Culture Intake		Survival Time (days)	Body Weights (g)			Liver Wt. (g)	Liver Damage	
		Total (g)	Av./Day (g)		Init.	Max.	Final		(mac.)	(hist.)
4a	4	527	74	7	114	114	102	—	Nil	—
7a	7	512	57	9	114	114	86	6.8	Mild	+
11a	11	972	70	14	110	116	105	8.5*	Sev.	+
14a	14	788	61	13	122	137	98	9.0*	Sev.	+

* Liver heavier than normal.

All guinea pigs had good appetites for the first seven days and ate the equivalent of about 70 g daily. The intake of pig No. 4a averaged 74 g daily until it died on the seventh day. Feed consumption for the three pigs fed material incubated for 7, 11, and 14 days dropped markedly 2–3 days before they died. None of the pigs survived the 28-day test period; all died within 7–14 days. Since this experiment commenced at the same time as that with culture material, all extract-fed pigs died before any culture-fed pigs.

Pig No. 4a, fed material incubated for 4 days, died on its seventh day but had a macroscopically normal liver. The pig fed material incubated for 7 days died on the ninth day, 29 August 1958, and was actually the first guinea pig fed material of fungal origin to exhibit macroscopic liver damage characteristic of the lesions produced in the livers of guinea pigs fed toxic grass. The specificity of eczema liver lesions was confirmed by histological examination. As some 20% of the liver surface area showed mottled light-brown tissue the damage was graded as mild. Because the liver of pig No. 11a was uniformly pale and slightly enlarged it was graded as severely damaged. The liver of Pig No. 14a was also graded as severe for it was similar in appearance and size to that of No. 11a and had numerous small (2 mm dia.) necrotic areas. Evans *et al.* (1957) stated that these necrotic lesion symptoms are a characteristic feature of facial eczema in the guinea pig. The body-weight data of the three pigs that exhibited liver damage indicated that pig No. 7a lost weight from the commencement of the feeding trial and that pigs Nos. 11a and 14a initially made slight gains in weight but finally lost weight. Maximum body weights for pigs Nos. 11a and 14a were attained by the ninth and seventh days of feeding respectively.

It is of interest to note that severe liver damage was obtained with the four-day-old cultures in contrast to the negative results from the test of ether-extracted material of the same age fed on a higher level. The culture-fed pigs Nos. 4 and 11 died after consuming only 335 g and 475 g respectively of culture, whereas with the extract procedure 512 g of culture was the minimum lethal dose (pig No. 7a). The ether-extracted material was fed at approximately three times the level of the cultures and this fact could account for the shorter survival times for the three pigs fed the liver-damaging extracts.

Perrin (1957) has reported results obtained from feeding guinea pigs on the most toxic grass then available. Visible liver damage was not observed before ten days, hepatomegaly was seldom seen before 21 days, and the earliest death occurred only after toxic grass had been fed for 20 days, followed by non-toxic grass for a further 24 days.

The results in Table 3 show that liver damage and death occurred on the ninth day and that hepatomegaly was detected as early as 13 days. The combined results of these feeding tests indicated that *S. bakeri* cultures incubated for four and after 14 days on a potato-carrot extract agar medium contained a concentrated source of a toxin capable of producing liver damage in the guinea pig similar to those found with toxic grass.

Tests of Extracts at Lower Feeding Level

The deaths of the extract-fed pigs after a much shorter feeding period than was expected when the experiment was planned, left sufficient extract material available for further studies. The material was fed at a lower level using, where possible, a larger number of guinea pigs. The daily feeding level was equivalent to 20 g of culture. Fed at this level the pigs were expected to live longer. The results are presented in Table 4.

TABLE 4. TESTS OF EXTRACTS AT LOWER FEEDING LEVEL

Pig No.	Incub. Time (days)	Culture Intake		Survival Time (days)	Body Weights (g)			Liver Wt. (g)	Liver Damage	
		Total (g)	Av./Day (g)		Init.	Max.	Final		(mac.)	(hist.)
4b	4	337	17	21	98	121	102	8.8*	Sev.	+
4c	4	290	18	16	115	128	100	6.9	Sev.	+
4d	4	356	17	27	122	140	117	7.2	Sev.	+
7b	7	176	20	10	103	126	120	6.0	Nil	+
7c	7	361	17	28	118	147	147	15.3*	V. sev.	+
7d	7	347	17	28	116	145	117	13.8*	V. sev.	+
11b	11	409	19	27	110	151	151	23.1*	V. sev.	+
14b	14	338	20	18	116	126	95	5.3	Mod.	+

* Liver heavier than normal.

At this lower feeding level of extract the guinea pigs in general lived longer, but survival times varied considerably for pigs similarly fed.

Three animals fed the extract of the four-day-incubated material survived for 21, 16, and 27 days. All showed severe macroscopic liver damage typical of the facial-eczema symptoms. Specificity of the liver lesions was confirmed histologically in each case. This material had previously failed to produce macroscopic liver damage when fed at a high level (Table 3). After initial gains all animals lost weight. With the seven-day-incubated material one animal (7b) died after only 10 days feeding. The liver of this pig showed no macroscopic changes but proved positive under the microscope. Although two other pigs (7c and 7d) completed the test period of 28 days, their growth was retarded and both showed very severe macroscopic liver lesions. The remaining 11- and 14-day-incubated material was sufficient for single pigs only. Guinea pig 11b survived 27 days and showed very severe liver lesions, whereas pig 14b died after 18 days with liver damage assessed as only moderate. The data are obviously inadequate because of animal variability to assess the relationship of incubation time with toxicity.

Efficiency of Extraction Procedure

Since liver damage resulted from feeding both cultures and ether extracts prepared from the cultures, it was of interest to determine whether the extraction procedure used had removed all the toxin or whether some toxin remained in the ether-extracted residues.

Ether-extracted residues were fed at a daily level of 25 g; feeding procedures were the same as for the cultures. Only two samples were available for testing. The results are shown in Table 5.

Ether-extracted residue derived from the 11-day-incubated material produced no liver damage, whereas that derived from the 14-day sample produced very severe liver damage. The two ether extracts of which these samples were the residue had both produced liver damage (Table 3). Because of the high level of toxicity in the material fed as culture it appeared unnecessary to use the extract procedure in order to achieve high-level consumption of fungal material. The use of the raw culture material also obviates the risk of losing some or all of the toxin in extraction.

TABLE 5. TESTS OF ETHER-EXTRACTED RESIDUES

Pig No.	Incub. Time (days)	Culture Intake		Survival Time (days)	Body Weights (g)			Liver Wt. (g)	Liver Damage	
		Total (g)	Av./Day (g)		Init.	Max.	Final		(mac.)	(hist.)
11r	11	860	25	35	189	314	314	10.2	Nil	—
14r	14	794	24	33	116	150	120	14.6*	V. sev.	+

* Liver heavier than normal.

Tests of Soil Bureau Cultures

Concurrently with the first feeding tests (Tables 2 and 3) material grown at the Soil Bureau on potato-carrot liquid media for 11 days was also tested as fungal cultures and as extracts of cultures. Cultures and ether extracts of cultures were prepared and fed as previously described. The results are shown in Table 6.

TABLE 6. TESTS OF SOIL BUREAU EXTRACTS AND CULTURES

Pig No.	Feeding Basis	Culture Intake		Survival Times (days)	Body Weights (g)			Liver Wt. (g)	Liver Damage	
		Total (ml)	Av./Day (ml)		Init.	Max.	Final		(mac.)	(hist.)
11f	25 ml cult	297	21	14	137	138	120	6.5	Mod.	+
11g	25 ml cult	448	21	21	136	143	129	9.8*	Sev.	+
11c	80 ml extr	395	56	7	104	119	119	3.9	Nil	—
11d	80 ml extr	420	52	8	107	123	123	4.2	Nil	—
11e	80 ml extr	696	58	12	100	131	131	—	Nil	—

* Liver heavier than normal.

When two pigs were fed the cultures direct at a daily level of 25 ml, pig 11f died on the 14th day (1 Sep 1958) and pig 11g died on the 21st day. These two pigs showed macroscopic eczema lesions which were histologically confirmed in each instance.

Three pigs, Nos. 11c, 11d, and 11e, fed the extracts equal to 80 ml of culture, survived for 7, 8, and 12 days respectively. Growth was slightly below normal for Nos. 11c and 11d, but all animals had macroscopically normal livers. Absence of liver damage could have been due to loss of the toxin in the preparation of the ether extracts. Other unknown causes appear to have been the reason for the premature death of these pigs.

Tests of Mycelium and Conidia for Toxin

Concurrently with the other experiments aimed at testing the fungal cultures (spore-bearing mycelium and media), preliminary attempts were made to test separately the mycelium and conidia for the presence of the toxin.

To encourage hyphal growth without sporulation, *S. bakeri* was grown on glucose broth media (100 ml per Roux flask) for only seven days at an incubation temperature of 24°C. Macerated fungal material mixed with 25 g ground non-toxic grass was offered to one guinea pig at a level of 50 ml daily for a 35-day test period, while a further pig was fed an ether extract of the fungal material equal to 180 ml daily.

In order to test a pure sample of conidia for the presence of the toxin, *S. bakeri* was cultured on potato-carrot-agar medium (20 g each per litre) in fifteen 6-in. petri dishes, incubated for 14 days at a temperature of 24°C. Spores were then washed off with 70 ml of sterile

water. One guinea pig was fed daily 2 ml of the spore suspension mixed with ground non-toxic grass for a 35-day test period. All test feeds were stored at 0–5°C. The results are shown in Table 7.

TABLE 7. TOXICITY OF MYCELIUM AND CONIDIA

Pig No.	Fungal Material	Culture Intake		Body Weights (g)			Liver Wt. (g)	Liver Damage	
		Total (ml)	Av./Day (ml)	Init.	Max.	Final		(mac.)	(hist.)
7e	Mycelium	1554	44	119	220	220	7.6	Nil	—
7f	Mycelium	3584	171	105	213	213	6.6	Nil	+
14c	Conidia			124	190	190	7.7	Mod.	+

All three guinea pigs completed their test-feeding periods. Pig No. 7e which was offered daily 50 ml of fungal culture averaged a daily intake of 44 ml. Pig No. 7f offered extracted material at a daily level of 180 ml averaged a daily intake equivalent to 171 ml of culture. Live-weight gains and liver weights were normal for both of these pigs. Pig No. 7e had a normal liver but the macroscopically abnormal liver of pig No. 7f on histological examination was found to have facial-eczema lesions. No spores were found in these cultures. It was apparent that *S. bakeri* grown on glucose broth media for seven days at 24°C contained the toxin in extremely low concentration. This preliminary experiment indicated that the toxin could be present in the mycelium. Pig No. 14c grew more slowly than the other two pigs but also had a normal-sized liver. The liver however exhibited moderate eczema damage which was confirmed histologically. This finding showed that the toxin was present in the conidia.

Tests of Different Strains on Different Media

A number of additional *S. bakeri* cultures were forwarded from the Soil Bureau for testing (Thornton and Ross 1959). These cultures represented strain C (high-sporing strain obtained from Claudelands mycelium), strain BS (isolated from spores observed at Claudelands) and strain G (isolated from hyphae on grass at Palmerston North). Each strain was grown on casamino acid-glucose, and on Raulin-Thom-sucrose media. Each culture contained equal quantities of material incubated for 4, 7, 11, 14, and 21-day periods which were bulked for testing purposes. These test cultures were fed at a daily level of 50 ml with 25 g ground non-toxic grass for 35 days. The results are shown in Table 8.

There was considerable variation in the intakes of the guinea pigs fed the different cultures. The pigs fed strains BS and G grown on casamino acid completed the test-feeding period and showed normal livers. The animal fed strain C grown on the same media survived 34 days and showed mild to moderate liver damage which was subsequently confirmed histologically. It also had an enlarged liver and had started to lose weight.

TABLE 8. TESTS OF STRAINS AND MEDIA

Fungal Strain	Media	Culture Intake		Survival Time (days)	Body Weights (g)			Liver Wt. (g)	Liver Damage	
		Total (ml)	Av./Day (ml)		Init.	Max.	Final		(mac.)	(hist.)
C	Casamino Acid	1216	36	34	124	162	141	11.7*	Mild-mod	+
BS	"	1563	45	35	143	212	212	7.0	—	—
G	"	1456	42	35	134	218	218	7.7	—	—
C	Raulin-Thom	1280	37	35	163	198	164	5.0	—	—
BS	"	836	36	23	169	188	178	12.1*	Sev.	+
G	"	1538	44	35	136	232	232	8.8	Nil	—

* Liver heavier than normal.

Although the pig fed strain C grown on Raulin-Thom lost considerable weight during the latter stages of the test feeding period it had a normal liver. Strain G grown on Raulin-Thom produced an abnormal liver but the symptoms were not confirmed by histology to be consistent with eczema damage. However the guinea pig fed strain BS grown on Raulin-Thom survived only 23 days. It had severe macroscopic symptoms of facial-eczema liver damage which were confirmed by histology. The liver was enlarged and although this animal grew slowly initially it lost weight over the later stages.

These preliminary tests indicated that strain C grown on casamino acid media and strain BS grown on Raulin-Thom media were toxic under the cultural conditions employed.

Strain and media appear to be important in determining toxin production. Obviously much more work on strain, media, and incubation time are necessary to develop methods for the production of maximum toxicity.

Production of Facial Eczema in Sheep

Since facial eczema is primarily a disease of ruminants it was essential to reproduce the complete facial-eczema syndrome in sheep by feeding them cultures of *S. bakeri*. In September 1958, suckling lambs approximately six weeks old and weighing 30–36 lb were drenched twice daily with macerated cultures. As facial eczema has never been detected in lambs in the spring it was considered safe for these experimental lambs to continue grazing with their dams. To facilitate drenching, agar cultures were liquified with water. Lambs No. 1–5 inclusive received a mixture of potato-carrot-agar cultures comprising approximately equal amounts of material incubated for 4, 7, 11, and 14 days. Lamb No. 6 received liquid potato-carrot cultures incubated for 7 days. Table 9 shows the daily feeding level, the length of time that the cultures were fed, and the resultant liver damage.

TABLE 9. FEEDING TESTS WITH LAMBS

Lamb No.	1	2	3	4	5	6
Daily feed level (g)	100	200	200	600	600	600 ml
No. days fed	7	7	7	7	7	21
Liv. dam (mac.)	—	—	—	V. mild	V. mild	Sev.
Liv. dam. (hist.)	—	?—	?—	+	+	+

When slaughtered after 7 days feeding the livers of lambs Nos. 1, 2, and 3 appeared normal to the naked eye. Liver sections from lamb No. 1 when examined histologically were found to be normal whereas similar liver sections from lambs Nos. 2 and 3 showed some degenerative changes in the epithelial cells of medium-sized bile ducts suggestive of early facial-eczema liver damage. Lambs Nos. 4 and 5 fed daily 600 g of culture for 7 days and then slaughtered showed liver damage. The liver of lamb No. 4 had one eczema-like lesion approximately one inch in diameter on the visceral surface near the junction of the cystic duct with the bile duct whereas the liver of lamb No. 5 had a lesion within the substance of the left lobe. Histological examination of sections of these lesions confirmed that they were characteristic of facial-eczema liver lesions as described by McFarlane *et al.* (1959).

As four days has been the shortest time at which eczema liver damage has been observed in grazing lambs after exposure to toxic pastures at Ruakura, it was probably not surprising that very mild liver damage was detected after only 7 days feeding of fungal cultures to two of these lambs. To allow sufficient time for full development of the disease symptoms, lamb No. 6 was dosed for 21 days with 600 ml daily of 7-day-incubated cultures and was slaughtered after 35 days. By the 16th day this lamb had developed thickened drooping ears and a scabrous muzzle, and was photosensitive. A blood sample gave a Van den Bergh reaction of over 2 mg/100 ml. Post-mortem examination showed that all tissues and organs throughout the body were mildly jaundiced. The only other significant feature was the liver which was a uniform light tan colour with normal consistency. Both the cystic and common bile ducts were occluded by fibrous tissue and sectioning of the liver revealed generalised fine fibrosis. The specificity of eczema lesions was confirmed by histological examination. This animal thus showed the complete facial-eczema syndrome.

DISCUSSION

The results presented demonstrated that cultures of the fungus *S. bakeri* are responsible for facial eczema in sheep.

The discovery of the beaker-test substance in the spores of *S. bakeri* focused attention on this previously undetected species of fungus and in particular on the sporulating phase. The presence of *S. bakeri* mycelium as well as spores with toxic grass suggested that this fungus was associated with toxic grass. Poor-sporulating cultures of *S. bakeri*

gave a negative beaker test and were not toxic. A high-sporing culture was obtained by selection of strain and media (Thornton and Ross 1959); such cultures gave positive beaker tests and were toxic.

The experiments to test *S. bakeri* cultures for toxicity were regarded as preliminary trials to test solely for the presence of the eczema toxin. Early death and the specific nature of the liver damage of guinea pigs fed extracts indicated an unexpectedly concentrated source of the eczema toxin compared with toxic grass. Animals fed less-toxic material lived longer and gave varied responses. Animal variation does not permit any precise conclusions to be made about factors affecting levels of toxicity. Indeed, some of the non-toxic cultures when re-tested may prove toxic. Under the cultural conditions employed, *S. bakeri* cultures grown on a potato-carrot medium for 4 and up to 14 days were highly toxic. All these cultures had sporulated. The detection of toxicity in both mycelium and spores and their association with toxic pastures reveals two ways in which the toxin could be ingested by the grazing animal. Quantitative assessments of the intensity of the beaker test, spore numbers, and the degree of liver damage are necessary to define their relationships. This discovery opens up a new field of research into problems of animal health.

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THE YIELD AND COMPOSITION OF MILK FROM SOWS FED VARYING PROPORTIONS OF SEPARATED MILK AND CONCENTRATES

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Summary

The results of two experiments are reported, in which the effects of three rations upon the yield and composition of sows' milk and the efficiency of production over the last seven weeks of an eight-week lactation were measured.

In the first experiment, conducted in the summer, a ration consisting of 40% separated milk, plus 60% concentrates was compared with a ration of separated milk alone. In the second experiment, conducted in the spring, a milk plus concentrate ration was compared with one of concentrates alone.

Milk only and milk plus concentrates were equally efficient in supporting milk production, and both were more efficient than concentrates alone.

The average milk energy production per sow and the mean gross efficiency of milk production for the sows on the four treatments were—Summer Experiment: Milk plus concentrate 313 therms,* 47.2%; Milk only 341 therms, 47.6%. Spring Experiment: Milk plus concentrate 383 therms, 53.2%; Concentrates only 308 therms, 44.3%.

Milk energy production was more efficient in the spring than the summer, while the efficiency of feed conversion by the litters was higher in the summer than in the spring. It is suggested that these are probably environmental temperature effects; temperatures conducive to efficient litter growth being antagonistic to efficient milk production.

There was a pronounced tendency for sow milk and creep energy intake to be inversely related.

The absolute level of milk production achieved in these experiments may have been influenced by the level of feeding imposed. This possibility will be further investigated.

INTRODUCTION

In a previous study of sow milk production (Smith 1952) it was found that the yields recorded were considerably higher than those reported by overseas research workers. It was suggested that separated milk, which constituted 40% of the Ruakura sow rations, might support a higher level of milk production than the concentrate rations used in other countries.

The two experiments reported in this paper were designed primarily to test this hypothesis. The first experiment involved the comparison of two rations, one consisting of 40% milk plus 60% concentrate, the other milk only. In the second experiment a 40% milk plus 60% concentrate ration was compared with concentrates alone.

* One therm = 1000 kilocalories.

These experiments are of some practical importance to commercial pig production in New Zealand. To make the most efficient use of separated milk throughout the dairy season, which extends from July to the end of the following May, some sows must be farrowed during winter so that weaned pigs are on hand to consume the rapidly increasing supply of milk in August and September. This programme necessitates the use of concentrates for sow feeding over a period of six to eight weeks, from May to July, before the commencement of the dairying season. Sows farrowing in July and August are generally fed on a mixture of milk and concentrates, while those farrowing from August to December receive a ration consisting solely of separated milk during lactation.

The three treatments under comparison are, therefore, representative of the types of rations in use in the industry and an indication of their relative efficiency in supporting lactation is of some importance.

TABLE 1. ANIMALS AND TREATMENTS

Season: Summer.

Treatment: 40% Separated Milk, 60% Concentrate.* Milk Only.

Sow	Litter No.	No. in Litter	Sow	Litter No.	No. in Litter
101	1	5	106	1	3
102	1	3	107	1	4
103	2	10	108	2	9
104	4	10	109	4	7
105	4	4	110	4	12
		32			35
		Mean 6.4			Mean 7.0

Season: Spring.

Treatment: 40% Separated Milk, 60% Concentrate. Concentrate only.†

111	1	7	115	1	7
112	1	7	116	1	5
101	2	6	118	2	5
107	2	4	117	2	6
113	2	8	110	5	6
114	2	6			
108	3	6			
		44			29
		Mean 6.28			Mean 5.8

* 3 parts barley, 1 part 60%-protein meat meal.

† 2 sows originally included in this group were discarded through sickness.

EXPERIMENTAL MATERIALS AND METHODS

The sows used, the number of pigs suckled, and the treatments imposed are shown in Table 1.

GENERAL MANAGEMENT OF STOCK

The experiments were conducted over two seasons. The rations, milk plus concentrates and milk only, were compared during summer when adequate separated milk was available, while the rations, milk plus concentrates and concentrates only, were studied in the early spring. Sows and litters, all of which were purebred Berkshire, were housed in 8ft x 8ft sties with a covered creep area along one side and a concrete feeding yard attached. Sows were fed three times a day. At each feeding the litter was removed from the pen until the sow had eaten her ration, to prevent confusion between the intakes of sow and litter. The quantities fed were based upon the number of piglets suckled. Originally it was planned to feed six units to each sow plus one unit per piglet suckled. However, under the very hot weather conditions encountered during the summer experiment, the first litter sows on the "milk only" treatment would not consume the required quantity of feed. All rations were therefore reduced to 4 units per sow plus $\frac{2}{3}$ units per piglet suckled.

Creep feed consisted of separated milk and a concentrate mixture (25% meat meal and 75% barley) fed in separate troughs and available at all times. Records of creep feed consumption were kept on a weekly basis. The sows were weighed daily, starting the day before the due farrowing date and continuing until 2 days after farrowing. They were then weighed once weekly until the 8th week when three successive weights were taken, on the 55th, 56th, and 57th days. Weights were taken at 7 a.m. prior to feeding and after the sows had been driven some distance to the scales to ensure that dunging and urination took place.

MILK YIELD MEASUREMENTS

Milk yield measurements were conducted once every 7 days and covered a period of 12 hours. The day of the week upon which the yield of any particular sow was determined depended upon the day she farrowed, the aim being to record production on the middle day of each of the eight lactation weeks. At 4 a.m. the sows whose yields were to be measured, were moved with their litters into a special building, the layout of which is shown in Fig. 1. The litters were then separated from their dams. Two sucklings without weighing were allowed at intervals which varied according to the stage of lactation, prior to the commencement of the sampling period. At 7 a.m. the piglets were weighed, allowed to suckle, reweighed and returned to their pens to wait for the duration of the appropriate suckling interval. This process was repeated until 7 p.m. The suckling intervals used were those determined by previous observational studies (Smith 1952) and were as follows: The first and second weeks, 60 min, the 3rd, 4th, and 5th weeks 65 min, the 6th week 70 min, and the 7th and 8th weeks 75 min.

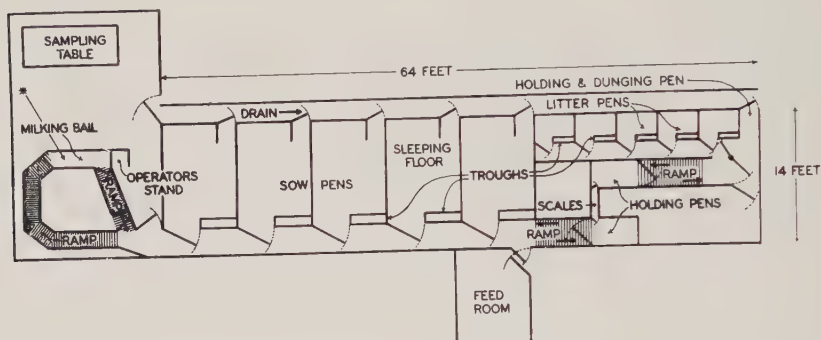


Fig. 1.—The design and layout of the building used for milk production studies.

* Direction of view of milking machine shown in Fig. 2.

The following morning the litters were again removed from the sows recorded the previous day and allowed to suckle twice at intervals as described above. At the time the third suckling would normally have occurred the sows were moved to a milking bail (Fig. 2) and were milked by machine. A sample was taken of the bulked milk of all functional glands, and analysed for fat, protein, and lactose.

It will be noted that the milk yield data presented in this paper relate to seven weeks of lactation and not to eight weeks. The first week has been omitted in all cases. Yield measurements were made

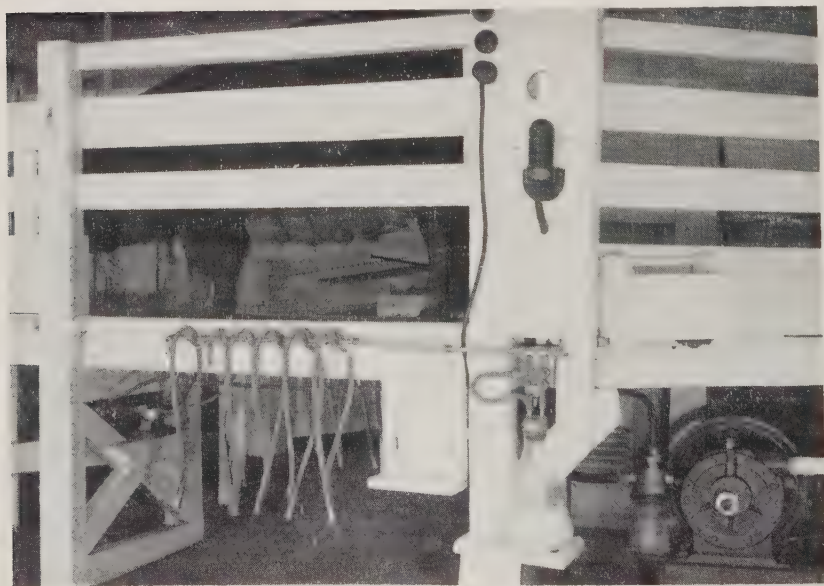


Fig. 2.—The raised milking bail and the milking machine used to obtain samples for analysis.

in the first week—on the 4th or 5th day after parturition, but samples were not taken for analysis. The rapid change in milk composition during the first week (Perrin 1955) rendered a sample taken on any one day of little value.

The building and the layout shown in Fig. 1 were planned to satisfy four requirements dictated by previous experience. These were:

1. To isolate the sows and litters from all other stock during the recording process.
2. To reduce the journey of the piglets from the holding pens to the scales, and from the scales to the sow, to a minimum.
3. To provide an efficient system of ensuring that the piglets dunged and urinated before each pre-suckling weighing.
4. To allow the sows out of their pens occasionally between sucklings without interference or disturbance to each other.

CALCULATION OF ENERGY VALUES

The feed intakes of sows and litters presented in this and subsequent papers were first calculated as pounds of total digestible nutrients (t.d.n.) using the expression suggested by Woodman (1957), $\text{t.d.n.} = \% \text{ digestible protein} + \% \text{ digestible carbohydrate} + \% \text{ digestible fibre} + (\% \text{ digestible oil} \times 2.25)$. Separated milk (8.5% dry matter) has been calculated as containing 0.77 lb. t.d.n. per gallon and the concentrate mixture 0.73 lb. t.d.n. per pound. These t.d.n. values were then converted to calories of metabolisable energy on the basis of 1814 kilocalories per pound of t.d.n. (Brody 1945). The gross energy (in kcal) of sows' milk has been calculated by multiplying the pounds of fat produced by 4132, pounds of protein by 2658, and pounds of lactose by 1792 (Andersen 1926). It has been assumed the metabolisable energy of sows' milk is 95% of the gross energy.

Comparisons of the effects of treatments have been based mainly upon the gross efficiency of milk production. The formula used was that of Brody (1945):

$$\text{Gross efficiency of milk production} = \frac{\text{Gross energy of milk produced (kcal.)}}{\text{t.d.n. of sows intake} \times 1814}$$

However, this formula makes no allowance for loss or gain in body weight during lactation. It was, therefore, necessary to form an estimate of the feed energy equivalent of a pound loss or gain in sow weight and adjust the denominator of the above expression accordingly. The calculations made were as follows:

Loss of weight. It was assumed that a pound of weight lost from a lactating sow was composed of depot fat and muscle in the ratio of 5:1 (Pomeroy 1941). This represents the ratio of change in the major tissues of a 328 lb pig on a sub-maintenance diet for 51 days—during which time a loss of weight of 65 lb was recorded.

It has been assumed (Hankins and Ellis 1945) that depot fatty tissue consists of 90% pure fat containing 3860 kcal/lb. It was

calculated from data from Armsby and Moulton (1925) that one pound of fat-free muscle would yield 590 kcal. One pound of live-weight loss was, therefore, credited with 3312 kcal.

In calculating the "intake equivalent" of sows which lost weight, 3312 kcal per pound of weight loss were added to the observed intake.

Gain in weight. It was assumed that a lactating sow fed in excess of maintenance and lactation requirements would use the excess directly for body gains. The gains made could, therefore, be regarded as the result of the productive portion of the ration and not subject to a reduction for maintenance.

If both gain and loss of weight by a lactating sow are considered to involve the same tissues, with the same energy value, namely 3,312 kcal, and if Armsby and Moulton's estimate that the energy recovered in gains represents 67% of the metabolisable energy intake above maintenance is accepted, then the energy intake above maintenance required to produce one pound of gain is 4,925 kcal or 2.7 lb of t.d.n. For the foodstuffs used in these experiments 2.7 lb of t.d.n. is equivalent to 3.5 gal milk or 3.7 lb meal. These two latter figures are very close to the value of 3.5 lb of meal reported by Woodman (1957) as being the meal requirement above maintenance to produce one pound of gain on a 300 lb pig.

In calculating the "intake equivalent" of the sows that gained weight, 4,925 kcal per pound of weight increase have been subtracted from the observed energy intake.

RESULTS

The milk production of the sows on the four treatments and the average fat, protein, and lactose content of the milk are shown in Table 2.

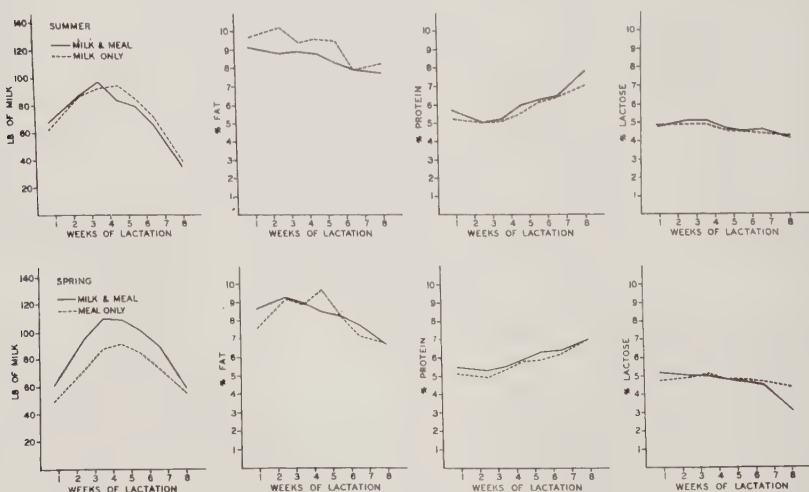


Fig. 3.—The average weekly milk yield and composition of the milk of the sows on the four treatments.

TABLE 2. PRODUCTION AND COMPOSITION OF MILK OF SOWS USED IN THE EXPERIMENTS*

Season: Summer

Treatment: Milk + Concentrates

Milk Only

Sow No.	Milk Yield (lb)	Fat %	Protein %	Lactose %	Sow No.	Milk Yield (lb)	Fat %	Protein %	Lactose %
101	361	8.9	5.8	4.7	106	222	9.1	5.6	4.7
102	284	8.2	5.9	4.6	107	326	8.7	5.6	4.7
103	718	9.4	5.5	4.7	108	814	9.2	5.5	4.6
104	897	8.0	6.0	4.7	109	556	8.5	5.6	4.7
105	412	8.3	5.3	4.9	110	847	10.7	5.5	4.4
Means: per sow	534	8.5	5.7	4.7		553	9.2	5.6	4.6
per piglet	83.5					79.0			

Season: Spring

Treatment: Milk + Concentrates

Concentrates Only

111	580	9.2	6.4	5.3	115	601	8.3	5.3	4.8
112	626	8.9	5.8	4.7	116	475	8.3	5.6	4.8
101	660	8.9	5.6	5.0	118	413	8.8	6.0	4.7
107	579	8.6	6.0	4.5	117	471	8.0	5.7	5.0
113	741	7.3	5.4	4.8	110	675	8.8	5.6	4.7
114	650	7.6	5.9	5.1					
108	685	8.9	6.1	4.9					
Means: per sow	645.8	8.5	5.9	4.9		527	8.4	5.6	4.8
per piglet	102.7					90.8			

* The data relate to the last 7 weeks of an eight-week lactation.

The average weekly yield and the average milk composition per sow in each of the four groups during lactation is shown in Fig. 3.

The raw data indicate that for the summer experiment the milk only and the milk and concentrate rations were equally effective as feeds for lactating sows. During the spring experiment the concentrate-only ration appears to have been less effective than the milk and concentrate mixture when compared both on a per-sow and a per-piglet-suckled basis, although the sows on the concentrate ration suckled fewer pigs. In general, while smaller litters usually result in lower total yield, the opposite is true of yield per pig (Bonsma and Oosthuizen 1935).

The changes in fat, protein, and lactose content of the milk followed the same trends as were found in previous work, but the fat content was higher and the protein and lactose content lower. These differences apparently exercised a compensatory effect since the energy content per pound of milk in the two studies is of the same order, namely 586 kcal/lb in the 1952 study, and 586, 617, 594, and 584 kcal/lb in the present groups.

A further and probably sounder comparison of the treatment effects is that based on the gross efficiency of milk production calculated from the expression:

Gross energy of milk produced (kcal)

t.d.n. intake of sow $\times 1814 \pm$ energy equivalent of weight changes

These efficiencies are shown in Table 3.

A multiple regression analysis of the data involving litter size, feed energy intake equivalent, and the gross energy of the milk produced, indicated that, for the summer trial, there was no evidence of significant treatment effects upon any variable. Variation in milk energy yield was, however, strongly associated with variations in both feed energy and litter size. When allowance was made for differences in litter size, the regression of milk energy yield upon feed energy was not significant, the equation being (y = milk energy, x = feed energy).

$$y = 0.68x - 139 \pm 43 \text{ (13.2\%)} \quad 1$$

In the spring trial there was a significant relationship between milk production and treatment, the milk-plus-concentrate group being significantly superior to the concentrate-only group.

The regression equations for the two spring groups were:

$$\text{Milk plus concentrate: } y = 0.41x + 94 \pm 26 \quad . \quad 2$$

$$\text{Concentrate only: } y = 0.61x - 116 \pm 41 \quad . \quad 3$$

It was evident that when these two latter equations were compared with that of the summer trial, that the concentrate group was comparable with the two summer treatments. When these three treatments were combined there was no evidence of either treatment or season effects, the pooled regression being:

$$y = 0.67x - 143 \pm 41 \text{ (12.7\%)} \quad 4$$

When the spring milk-plus-concentrate group was included this was found to differ significantly:

$$y = 0.66x \quad \left. \begin{array}{l} - 81 \text{ spring milk + concentrate group} \\ - 135 \text{ the three other groups} \end{array} \right\} \pm 38 \text{ (11.1\%)}$$

After allowing for differences in feed energy equivalent, the milk energy output of the spring milk-plus-concentrate group exceeded that of the other three groups by 54 units, this difference being highly significant.

TABLE 3. GROSS EFFICIENCY OF MILK PRODUCTION OF ALL SOWS
IN THE FOUR GROUPS

Season: Summer

Treatment: Milk + Concentrate

Sow No.	No. of Pigs.	Av. Live Wt. of Sow	Gross Energy Milk (therms)*	Energy Intake (therms)	Loss or Gain in Wt. (lb)	Energy Intake Equiv.	% Efficiency
101	5	511	218	696	+20	598	36.4
102	3	291	164	397	+15	323	50.7
103	10	507	435	687	-41	823	52.8
104	10	380	515	792	-52	964	53.4
105	4	392	233	530	-24	609	38.2
Means		456	313		-18.4	663.4	47.2

Milk energy per piglet 48.9 therms.

Treatment: Milk Only

106	3	393	135	549	+24	431	31.3
107	4	350	193	499	-16	552	34.9
108	9	526	493	735	-43	877	56.2
109	7	451	323	630	-21	699	46.2
110	12	549	563	767	-78	1025	54.9
Means		454	341.4		-26.8	716.8	47.6

Milk energy per piglet 48.7 therms.

Season: Spring

Treatment: Milk + Concentrate

111	7	380	370	567	-27	656	56.4
112	7	372	382	571	-31	674	56.7
101	6	478	402	512	-63	720	55.8
107	4	422	344	441	-45	590	58.3
113	8	415	389	631	-46	783	49.7
114	6	387	372	530	-48	689	54.0
108	6	473	425	587	-48	746	56.0
Means		418	383.4		-44	694	53.2

Milk energy per piglet 61 therms.

Treatment: Concentrates Only

115	7	361	343	644	-36	763	45.0
116	5	362	274	470	-43	672	40.8
118	5	318	251	470	-27	559	44.9
117	6	381	268	567	-40	699	38.3
110	6	502	403	519	-78	777	51.9
Means		385	307.8		-46	694	44.3

Milk energy per piglet 53.1 therms.

* One therm = 1000 kilocalories.

In general it would appear that (i) there was no appreciable difference in the efficiency with which milk plus concentrate and milk only were converted to milk energy by the sow, (ii) that concentrates alone were less efficient than milk plus concentrates in supporting milk energy production, and (iii) that there was a seasonal advantage in favour of spring production.

In both seasons there was a significant association between feed energy intake equivalent and litter size, the relationship being unaffected by treatments.

Since the rationing system used was based on litter size this association was inevitable. The feed energy data do, however, include the energy equivalents of weight losses or gains, in addition to the rations actually fed. For this reason the regression of feed energy intake equivalent upon litter size should theoretically provide an estimate of the feed energy required to support a milk energy yield of the order achieved by the sows in these experiments without loss or gain in weight.

A joint analysis of both seasons' data indicated that the seasonal differences were not significant. From the pooled data the overall relationship between feed energy and litter size was:

$$(x = \text{feed energy, } z = \text{litter size})$$
$$x = 62.2z + 296 \pm 65 \quad 9.4\%$$

The regression coefficient being highly significant.

Efficiency of Energy Conversion by the Litter

The efficiency with which the sow converts her feed energy to milk for her young is only the first step in the process of litter production. The efficiency with which this milk, and the feed taken directly from the creep are utilised by the piglets also contributes to the practical efficiency of the process of lactation in the sow. In Table 4 are shown the average intakes and efficiencies of the litters from the sows in the four groups. One sow has been excluded from the spring milk-and-concentrate group because her litter suffered a set-back in the 6th and 7th weeks of life due to a respiratory infection.

It would appear that, whereas the cool environment in spring was more favourable for milk production than the hotter conditions of summer, the reverse was true for litter growth and efficiency. The cold conditions prevailing during the spring experiment when temperatures as low as 32°F were recorded inside the 8 ft × 8 ft farrowing units in which the sows and litters were housed, have resulted in a higher energy intake per pound of liveweight gain than was required by the summer litters.

The second point of interest is that the lower milk energy output of the concentrate-fed sows was compensated for by the increased creep intake of the litters.

The difference in creep intake between the litters of the sows on the milk-and-concentrate and milk-only rations was due to the small

TABLE 4. INTAKE OF ENERGY FROM SOW'S MILK AND CREEP AND EFFICIENCY OF ENERGY CONVERSION TO LIVE WEIGHT OF LITTERS FROM THE 4 GROUPS

Season: Summer

Treatment	Intake Energy per Pig (therms) from:			Gain per Pig (lb)	Therms per lb Gain
	Sow's Milk	Creep	Total		
Milk + Concentrate	46.5	34.1	80.6	38.1	2.12
Milk Only	46.3	28.1	74.4	34.4	2.16

Season: Spring

Milk + Concentrate	56.5	21.0	77.5	33.2	2.33
Concentrate Only	50.4	26.4	76.8	33.7	2.28

intake per pig of one sow, No. 110, in the milk-only group. When this sow was excluded, the average intake of sow's milk and creep was 47.1 and 34.1 therms respectively. It is obvious that the relationship between levels of sow milk and creep intake will merit close examination when more data become available.

GENERAL DISCUSSION

The original thesis advanced, that the inclusion of separated milk in the rations of lactating sows might support a higher level of milk production than a ration consisting of concentrates alone, is supported by the results of these experiments, at least in regard to the concentrate mixtures and the level of milk supplement employed. In general terms, the results indicate that to produce one therm of milk energy the concentrate fed sows required 2.25 therms of feed energy equivalent while the milk supplemented sows required 1.83 therms.

While these findings offer a partial explanation for the high yields of the sows used in a previous study (Smith 1952), as compared with those reported by other workers, they by no means completely resolve the problem.

The mean daily yield of the sows recorded at this station in 1951 was 14.4 lb whereas the mean yield of the sows receiving milk in the present study was 12 lb. Wells *et al.* (1939) recorded a mean production of 11.7 lb daily from 43 lactations, while Olafsson and Larsson (1930) reported mean daily yields of 10.3 lb. More recently Berge and Indrebo (1953) reported yields of 13.6 lb. It is apparent, therefore, that the level of production of the sows in the present experiments was of the same order as those reported by a number of overseas investigators. At the same time, the ration level of the present sows was only two-thirds of that received by the animals recorded in 1951.

There remain two possible explanations for the high level of production of the original sows in comparison with those of the present study and of overseas investigators:

1. The original sows were inherently superior milk producers.
2. The reduction of the ration in these experiments to two thirds of that fed to the original sows has resulted in a lowering of yield. This possibility will be explored in future experiments.

To return to the discussion of the finding that milk-supplemented diets appear to be more efficient in supporting milk production than concentrates, there are two reasonable suggestions that may be advanced in explanation:

- a. The heat increment or specific dynamic action of meal is greater than that of milk, which, if true, would result in a lower net energy per pound of metabolisable and digestible energy intake from the meal. This suggestion, however, is not supported by a difference in efficiency between the milk-only and milk-and-concentrate groups.
- b. The addition of milk to the concentrate ration improved the biological value of the protein and this resulted in better utilisation. This latter suggestion requires investigation.

The gross efficiency of milk production exhibited by the sows in these studies, 44%–53% is of the same order as that (45%) found by Lodge (1957). Compared with dairy cows these levels of efficiency are extremely high. Brody (1945) reported efficiencies of 28%–34% for cows of 1000 to 1300 lb live weight consuming 17–28 lb of t.d.n. and producing 28–50 lb of fat-corrected milk (f.c.m.) per day. The data from the present study are for sows weighing 350–550 lb, consuming an average of 7.5 lb of t.d.n. and producing 20 lb of f.c.m. daily.

The difference in efficiency of milk production between summer and spring is of particular interest and is probably related to environmental temperatures. There is ample evidence that the efficiency of feed utilisation by dairy cows is adversely affected by high environmental temperatures. Observation of sows during the summer months leaves one in no doubt as to the distress occasioned by temperatures of 75°F and above. Sows subjected to these conditions exhibit an accelerated respiration rate, in an attempt to increase respiratory evaporation. Wood (1926) has shown the effect of high temperatures upon pigs kept in metabolism crates to be an increase in the energy expended on muscular activity as a result of accelerated respiration, with a consequent increase in maintenance requirements.

Heitman and Hughes (1949) in a study specifically designed to measure the effects of temperature variations upon the growth rate and feed efficiency of swine, found that the optimum temperature for pigs between 70 and 140 lb live weight was 70°F while those between 160 and 260 lb performed more efficiently in an environmental temperature of 60°F. All this evidence suggests that during the summer months lactating sows should be housed in adequately ventilated quarters, and in addition some form of cooling device should be provided such as a wallow or water spray for use during the hotter part of the day.

Whereas the present data indicate that summer heat may depress the efficiency of milk production by the sow, it also suggests that winter cold reduces the efficiency of litter growth. It would appear, therefore, that since, of necessity, the sow and litter must be housed together, two different environments must be provided in the same structure.

A further point worth discussion arises from consideration of the ration level used in these experiments and the derived regression of feed intake on litter size. Apart from some discrepancies, which occurred at the commencement of the trials, the ration level was 4 units for the sow and $\frac{2}{3}$ units per pig suckled. The regression equation was, however, derived from "equivalent feed energy intake" data which included allowances made for losses and gains in body weight by the sow. The energy from body tissues was used by the sows to supplement the rations fed and for this reason the regression which, translated into feed units, was $x = 0.96z + 4.5$, is a more realistic expression of the feed requirements of sows whose yields are of the order of those used in these experiments. In practical terms, therefore, it would appear that a ration consisting of 4.5 units per sow and 1 unit per piglet suckled should, theoretically, support a milk production of approximately 12 lb per day with a minimum of weight loss by the sow.

In view of the plan to investigate further the effect of level of feeding upon milk yield, it is appropriate here to mention that the gross efficiency of the sows used in the spring 1951 experiment was 47%. As mentioned previously, these sows were fed at the rate of 6 units per sow and one unit per piglet suckled. It is possible that the discrepancy between this level of efficiency and that exhibited by the present spring milk-and-concentrate group may have been due to the difference in level of feeding and this aspect will be investigated further.

The final outcome of the reproductive cycle of the sow is a weaned litter and under commercial conditions the weight and condition of the litter is generally the criterion by which the sow ration is judged. In the present study, as shown in Table 4, the litter weight gains were materially affected by creep intake. This is particularly true of the litters from the sows fed only concentrates, which ate sufficient from the creep to make up for the lower milk yield of their dams, so that they weaned at the same weight as the pigs from the milk-plus-concentrate group. There is little doubt that there is, in general, an inverse relationship between milk yield and creep intake, but it is equally true that the vitality of the litter, the shape of the lactation curve, and probably other factors as well, materially affect this relationship. In view of the importance of creep feeding in the overall efficiency of litter production, the assessment of the factors that determine the level of creep intake is exceedingly important.

ACKNOWLEDGEMENTS

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THE YIELD AND COMPOSITION OF MILK FROM SOWS FED ON THREE RATION LEVELS

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Summary

Two experiments were conducted in which the effects of three ration levels upon the yield and composition of sows' milk and the efficiency of milk production over the last seven weeks of an eight-week lactation were examined.

The levels fed were: 6 units per sow and 1 unit per piglet suckled; 4 units per sow and $\frac{2}{3}$ per piglet; and 3 units per sow and $\frac{1}{2}$ per piglet per day. (A gallon of milk or a pound of concentrate was regarded as one feed unit.)

There was a slight reduction in yield per sow and per piglet when the ration was reduced from 6 + 1 to 4 + $\frac{2}{3}$, but no reduction when the ration was lowered from 4 + $\frac{2}{3}$ to 3 + $\frac{1}{2}$. The average milk yield per sow and per piglet for the 4 treatments were, in the first experiment: 6 + 1, 573 lb and 86.7 lb; 4 + $\frac{2}{3}$, 493 lb and 82.1 lb; and in the second experiment: 4 + $\frac{2}{3}$, 668 lb and 78.6 lb; 3 + $\frac{1}{2}$, 614 lb and 83 lb.

The reduction in yield resulting from lowered intake was largely offset by the increased energy content of the milk, the values for the treatments being, for the first experiment: 6 + 1: 619 kcal/lb; 4 + $\frac{2}{3}$: 638 kcal/lb; and for the second experiment, 4 + $\frac{2}{3}$: 648 kcal/lb; 3 + $\frac{1}{2}$: 653 kcal/lb.

The average yield of milk energy per sow and per piglet for the four treatments were, for the first experiment: 6 + 1, 354 and 53.6 therms*; 4 + $\frac{2}{3}$, 314 and 52.4 therms; and in the second experiment: 4 + $\frac{2}{3}$, 433 and 50.9 therms; 3 + $\frac{1}{2}$, 401 and 54.2 therms.

There was a pronounced tendency for the fat content of the milk to increase as the ration was lowered and for the lactose content to decrease.

To a large extent the sows offset the effect of lower rations by the use of body reserves.

The sows on the lower rations used their feed energy equivalent (allowance being made for the energy content of body reserves) more efficiently than those on the higher ration. The values for gross efficiency of milk production for the four treatments were: 6 + 1 : 40%; 4 + $\frac{2}{3}$: 48%; 4 + $\frac{2}{3}$: 48%; and 3 + $\frac{1}{2}$: 53%.

The calculated percentages of the metabolisable sow feed energy equivalent recovered as tissue energy in the litters were: 6 + 1 : 20%; 4 + $\frac{2}{3}$: 24%; 4 + $\frac{2}{3}$: 25%; and 3 + $\frac{1}{2}$: 26%.

INTRODUCTION

For many years in New Zealand, the recommended daily ration for lactating sows has been six feed units per sow and one unit per piglet suckled, one gallon of separated milk or one pound of concentrate being regarded as a feed unit. This standard was based on trial and error, and no experimental evidence exists to indicate the

* One Therm = 1000 kilocalories.

superiority of such a level of feeding over any other. Experience in sow feeding experiments at this Station has shown that when the above ration consists entirely of separated milk, the quantity involved often exceeds the appetite of the sow, especially in the case of first-litter sows, and sows suckling more than six pigs.

The problem is accentuated in the summer months when high environmental temperatures depress appetite. At this time of the year there is usually ample separated milk available for pig feeding, and commercial farmers are reluctant to reduce the bulkiness of the ration by replacing some of the milk with concentrates. In consequence many farm sows must consume a lower ration than that recommended.

The results of previous studies on sow milk production at Ruakura (Smith 1959) have suggested that sows fed less than the recommended level may produce less milk, but there was also an indication that they may utilise their feed more efficiently. The experiments reported here were designed to examine the effect of various levels of intake upon the yield and composition of sows' milk, and upon the efficiency of milk production.

EXPERIMENTAL MATERIALS AND METHODS

Two groups of five sows were used in each of two experiments in which three levels of intake were compared. While it would have been preferable to use 3 groups in a single experiment, the number of suitable animals available within each season, and the time-consuming nature of the work, necessitated the division of the investigation into two parts. In the first experiment, carried out in the summer, the recommended ration level (6 feed units per sow and 1 unit per piglet suckled) was compared with a ration of 4 units per sow and $\frac{2}{3}$ units per piglet. In the second experiment, conducted in the autumn, the comparison was between 4 units per sow and $\frac{2}{3}$ units per piglet, and 3 units per sow and $\frac{1}{2}$ unit per piglet suckled. The levels compared were, therefore, in the ratio of $1 : \frac{2}{3} : \frac{1}{2}$. For convenience these ration levels are referred to in the course of the paper as "6 + 1", "4 + $\frac{2}{3}$ ", or "3 + $\frac{1}{2}$ ". The daily feed consisted of 40% separated milk and 60% concentrate on a feed unit basis. The concentrate used was a mixture of 3 parts by weight of barley meal and 1 part of 60% protein meat meal.

The sows used and the vital statistics relevant to them are listed in Table 1.

The sows were housed in 8 x 8 ft sties with a feeding yard and creep attached. They were allowed access to pasture for half an hour morning and evening. Yield measurements were carried out over a 12-hour period on one day of each week of lactation.

Creep feed, which was fed to appetite, consisted of separated milk and meal (as for sows) fed in separate troughs, and was available from the end of the third week until weaning. The piglets did not have access to their dams' feed.

TABLE 1. LITTER NUMBER AND NUMBER OF PIGS SUCKLED BY SOWS ON THE FOUR TREATMENTS

Season: Summer

Treatment: 6 + 1			4 + $\frac{2}{3}$		
Sow No.	Litter No.	Pigs Suckled	Sow No.	Litter No.	Pigs Suckled
121	1	5	119	1	7
122	1	7	120	1	3
115	2	7	102	2	8
117	3	7	111	2	7
107	3	7	106	3	5
Totals		33			30
Mean		6.6			6.0

Season: Autumn

Treatment: 4 + $\frac{2}{3}$			3 + $\frac{1}{2}$		
123	1	8	124	1	6
116	2	8	112	2	8
114	3	9	113	3	10
108	4	9	101	3	7
		*	125	4	6
Totals		34			37
Mean		8.5			7.4

* One sow from this group was eliminated because of sickness.

Details of the techniques used in measuring sow milk yield and taking samples for analysis (together with a full description of the methods used in estimating the energy values employed) have been presented in a previous publication (Smith 1959).

The only calculations not already described were those involved in estimating the energy content of suckling pigs. This was calculated from data published by Berge and Indrebo (1954), a pig one week of age being credited with 753 kilocalories per pound liveweight, and a pig at 8 weeks of age, 1055 kilocalories per pound.

It will be noted that all the data presented relate to the last seven weeks of an eight-week lactation.

RESULTS

The yield and composition of the milk of the experimental sows are shown in Table 2.

TABLE 2. YIELD AND COMPOSITION OF MILK OF INDIVIDUAL SOWS ON THE 4 TREATMENTS

Season: Summer

Treatment: 6 + 1					4 + $\frac{2}{3}$				
Sow No.	Milk Yield (lb)	Fat %	Prot. %	Lact. %	Sow No.	Milk Yield (lb)	Fat %	Prot. %	Lact. %
121	458	9.8	6.5	4.8	119	497	9.6	6.1	4.6
122	367	9.0	6.4	4.8	120	189	8.7	6.6	4.8
115	614	8.7	5.7	5.1	102	720	9.6	6.0	4.7
117	645	8.8	6.1	5.0	111	606	9.5	6.3	4.7
107	779	8.6	5.9	4.9	106	452	9.2	5.9	5.0
Means per sow	573	9.0	6.1	4.9		493	9.3	6.2	4.7
Means per piglet	86.7					82.1			

Season: Autumn

Treatment: 4 + $\frac{2}{3}$					3 + $\frac{1}{2}$				
123	438	9.5	6.1	4.7	124	434	10.0	5.4	4.7
116	682	10.6	5.8	4.6	112	665	10.7	6.3	4.4
114	772	9.3	6.2	4.6	113	809	9.2	5.6	4.6
108	781	9.7	6.0	4.9	101	623	10.7	5.9	4.5
					125	542	10.0	5.7	4.6
Means per sow	668	9.8	6.0	4.7		614	10.1	5.8	4.56
Means per piglet	78.6					83.0			

The average weekly yield and composition of the milk of the four groups are shown in Figs. 1 and 2.

It would appear from the data in Table 2 and Figs. 1 and 2a that a reduction in ration level from 6 + 1 to 4 + $\frac{2}{3}$, and again from 4 + $\frac{1}{2}$ to 3 + $\frac{1}{2}$ has in each case resulted in a lower level of milk yield per sow.

The effect of litter size must, however, be taken into account. Normally, larger litters result in greater total yield per sow, but less milk per piglet suckled (Bonsma and Oosthuizen 1935). This effect is seen in the 4 + $\frac{2}{3}$ and 3 + $\frac{1}{2}$ treatments where an average of 8.5 and 7.4 piglets were suckled by the sows of the two groups respectively. Here the sows on the 3 + $\frac{1}{2}$ treatment yielded less milk per sow, but more per piglet. This was not true, however, for the 6 + 1 and 4 + $\frac{2}{3}$ treatments where the sows on the latter ration suckling fewer pigs produced less milk both on a per-sow and per-piglet basis.

The composition of the milk produced was apparently affected by the feeding levels imposed. There was a rise in fat content and a

lowering of the lactose content of the milk as the ration level was reduced.

Since the ration levels apparently affected both yield and composition, and in one case at least these two characters have been inversely affected, a more valid comparison of treatments can be obtained from the gross energy of the milk yields which combines measurements of both yield and composition. The gross energy of the milk produced by the sows in the two experiments is shown in Table 3.

TABLE 3. GROSS MILK ENERGY PER SOW AND PER PIGLET PRODUCED
BY SOWS ON THE 4 TREATMENTS
(Energy values in therms*)

Season: Summer

Treatment: 6 + 1				4 + $\frac{2}{3}$			
Sow No.	Gross Energy of Milk	No. of Pigs	Gross Energy per Pig	Sow No.	Gross Energy of Milk	No. of Pigs	Gross Energy per Pig
121	304	5	60.8	119	319	7	45.6
122	231	7	33.0	120	117	3	39.0
115	369	7	52.7	102	461	8	57.6
117	399	7	57.0	111	391	7	55.8
107	468	7	66.8	106	284	5	56.8
Means	354	6.6	53.6		314	6.0	52.4
Kcal. per lb of milk			619				638

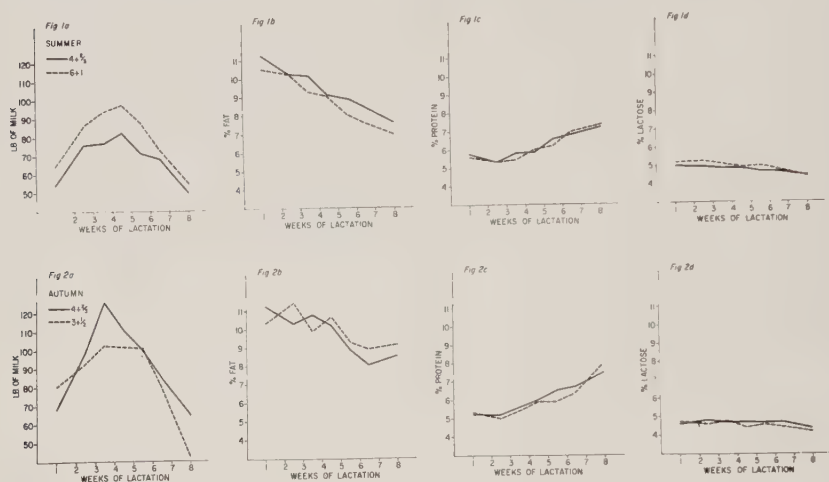
Season: Autumn

Treatment: 4 + $\frac{2}{3}$				3 + $\frac{1}{2}$			
123	279	8	34.9	124	279	6	46.5
116	460	8	57.5	112	459	8	57.4
114	489	9	54.3	113	497	10	49.7
108	504	9	56.0	101	421	7	60.1
				125	351	6	58.5
Means	433	8.5	50.9		401	7.4	54.2
Kcal per pound of milk			648				653

* One therm = 1000 kilocalories.

Comparison between the 6 + 1 and 4 + $\frac{2}{3}$ treatments on the basis of gross energy production per sow and per piglet still shows the 4 + $\frac{2}{3}$ ration level to have supported a slightly lower yield. On the other hand both the 4 + $\frac{2}{3}$ and 3 + $\frac{1}{2}$ treatments in the autumn experiment yielded more milk energy per sow than the 6 + 1 group in the summer and, in addition, the 3 + $\frac{1}{2}$ treatment yielded more milk energy per pig than the 6 + 1 group.

The failure of low levels of feed intake to depress milk energy production drastically was partly due to the use made of body reserves by the sows on the lower rations, as is shown in Table 4.



Figs. 1 and 2.—The effect of stage of lactation upon the average yield and composition of the milk of the sows on the four treatments.

In the first experiment, 6 + 1 versus 4 + 2/3, the mean weight losses per sow were 29 and 37 pounds respectively. Sow No. 120, in the 4 + 2/3 group only suckled 3 pigs and gained weight in the course of lactation. It may be assumed that for the number of pigs suckled and for the level of production exhibited by this sow, the 4 + 2/3 ration was more than adequate and the ration level *per se* had no effect upon her milk yield. When this sow is excluded, the mean weight losses were 29 lb for the 6 + 1 group suckling 6.6 pigs per litter, and 50 lb for the 4 remaining sows in the 4 + 2/3 group suckling 6.7 pigs per litter. In the second experiment, the average litter size in both groups was greater than in the first experiment and the sows on both treatments lost substantially more weight, the losses being 79 and 95 lb for the 4 + 2/3 and 3 + 1/2 levels respectively.

The sources of the energy upon which the sows in the four groups operated were as shown in Table 5.

In addition to the use of body reserves to offset a lower daily feed intake, the sows on the 4 + 2/3 and 3 + 1/2 rations utilised the available energy more efficiently than the sows on the 6 + 1 ration. In round figures, the gross efficiencies of milk production for the four groups were 40%, 48%, 48% and 53% for the 6 + 1, 4 + 2/3, 4 + 1/3 and 3 + 1/2 ration levels respectively.

These efficiency values are, however, not absolutely accurate, especially those for the summer experiment, since both the 6 + 1 and 4 + 2/3 ration sows had access to grass for an hour each day. As a result, the order of efficiency indicated in Table 4 may be too high.

TABLE 4. GROSS EFFICIENCY OF MILK PRODUCTION OF SOWS ON THE 4 TREATMENTS (energy values in therms)

Season: Summer

Treatment: 6 + 1

Sow No.	Mean Wt. (lb)	Loss or Gain in Wt. (lb)	Energy Equiv. of Wt. Changes	Energy of Feed Intake	Total Energy Intake Equiv.	Milk Energy Produced	Gross Efficiency %	Therms Feed/Eq. Therms Milk
121	364	— 16	51	665	716	304	42.46	2.36
122	315	— 9	22	726	748	231	30.88	3.24
115	432	— 21	70	839	909	369	40.59	2.46
117	427	— 33	109	836	945	399	42.22	2.37
107	457	— 67	222	861	1083	468	43.21	2.31
Mean per sow	399	— 29.2	94.8	785.4	880.2	354.2	39.87	2.55

Treatment: 4 + $\frac{2}{3}$

119	316	— 22	73	570	643	319	49.61	2.02
120	381	+ 14	71	399	328	117	35.67	2.80
102	396	— 90	298	547	845	461	54.56	1.83
111	448	— 56	186	568	754	391	51.86	1.93
106	463	— 33	109	483	592	284	47.97	2.08
Mean per sow	401	— 37.4	119.0	513.4	632.4	314.4	47.93	2.13

Season: Autumn

Treatment: 4 + $\frac{2}{3}$

123	298	— 27	109	581	690	279	40.43	2.47
116	431	— 73	242	609	851	460	54.05	1.85
114	423	—102	338	655	993	489	49.24	2.03
108	506	—114	378	649	1027	504	49.07	2.04
Mean per sow	414.5	— 79.0	266.75	623.5	890.25	433.0	48.20	2.10

Treatment: 3 + $\frac{1}{2}$

124	421	— 61	202	392	594	279	46.97	2.13
112	422	—107	354	455	809	459	56.74	1.76
113	416	—111	368	538	906	497	54.86	1.82
101	478	— 87	288	440	728	421	57.83	1.73
125	443	—109	361	387	748	351	46.93	2.13
Mean per sow	436	— 95.0	314.6	442.4	757.0	401.4	52.67	1.91

Both groups, no doubt, obtained some nutrients from the pasture despite the short period allowed for grazing. It is also possible that the sows on the lower ration, being hungrier, grazed more avidly and obtained relatively more nutrients than their better-fed mates.

It seems unlikely, however, that the large difference in efficiency shown could all be due to differential grass intake. If it is assumed that the sows on the 4 + $\frac{2}{3}$ ration ate 4 lb of grass in the hour spent grazing (data from experiments conducted at this Station indicate an intake of 26 lb of grass daily for sows permanently on pasture (Bal-

TABLE 5. PERCENTAGES OF SOW FEED-ENERGY EQUIVALENT DERIVED FROM BODY RESERVES AND ACTUAL FEED INTAKE RESPECTIVELY

Treatment	Season: Summer		Autumn	
	6 + 1	4 + $\frac{2}{3}$	4 + $\frac{2}{3}$	3 + $\frac{1}{2}$
Source of energy				
Body reserves	11%	23%	30%	42%
Actual feed	89%	77%	70%	58%

linger 1939)), and that the t.d.n. value of the pasture was 11.8 per hundred pounds, the efficiency of this group of sows would be reduced from 48% to 45%, a value still well in excess of the 40% shown by the 6 + 1 ration sows. Since the 6 + 1 sows would have eaten some grass, the margin between the relative efficiencies would not be decreased to the extent shown.

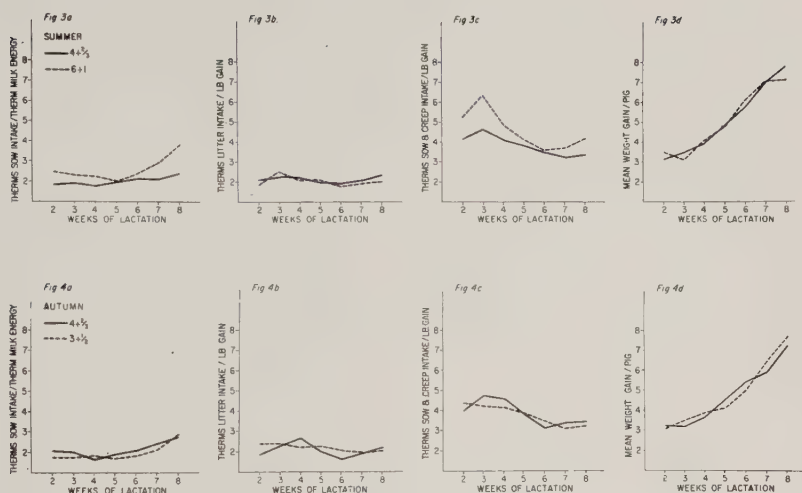
In the second experiment, conducted during March and April, pasture intake was negligible. The sows concerned occupied the pens vacated by the animals used in the first experiment. At this time no green grass was left on the paddocks since no rain had fallen for a month. The sows were farrowed early in March and no appreciable rain fell during the course of the experiment.

The gross efficiencies of milk production shown in Table 4 for the sows on the 4 + $\frac{2}{3}$ and 3 + $\frac{1}{2}$ treatments are, therefore, as accurate as the experimental techniques would allow.

Other Measures of Efficiency

The gross efficiency of milk production for the whole lactation, discussed in the previous section, is shown on a weekly basis in Figs. 3a and 4a. The sows fed on the 4 + $\frac{2}{3}$ ration were consistently more efficient than the sows on the 6 + 1 ration (3a). Again, the sows on the 3 + $\frac{1}{2}$ ration were more efficient than those on the 4 + $\frac{2}{3}$, in all but the 4th week (4a).

Figs. 3b and 4b show the metabolisable energy intake of sows' milk and creep per pound of live-weight gain for the litters of the sows in the 4 groups. The sow milk intakes of the litters are those shown in Table 3, less the amount of milk taken from the sow by machine during sampling, the remainder then being converted to metabolisable energy. The energy requirement per pound of gain was approximately 2.1 therms, and the ration level of the sows did not have any consistent effect upon the efficiency of energy conversion by the litters. Figs. 3c and 4c show the relationship of the feed energy equivalent available to the sow and to the litters in the creep, to the gains made by the litters. The overall efficiency of feed energy utilisation increased throughout lactation, except in the case of the 6 + 1 group during the last two weeks, despite a concurrent decrease in the efficiency of milk production. This contra effect was due to the very efficient use made by the litters of feed taken directly from the creep and to the rapid increase in the consumption of creep feed energy. The mean weekly weight



Figs. 3 and 4.—Three measures of the efficiency of feed energy utilisation by the sows and litters of the four groups, and the weekly weight gains of the litters.

gains per pig are shown in Figs. 3 and 4d and it is apparent that the treatments to which the sows were subjected had little effect upon the growth of the litters. The growth of the litter is, however, a result of both sow milk and creep energy intake, and there is often an inverse relationship between the amounts consumed from these two sources of energy. The mean intakes of milk and creep energy per pig are shown in Table 6.

TABLE 6. AVERAGE INTAKE OF METABOLISABLE MILK AND CREEP ENERGY PER PIG AND GAIN PER PIG OF LITTERS FROM SOWS ON THE 4 RATION LEVELS (energy values in therms)

Treatment	No. of Sows	No. of Pigs	Energy Intake per Pig from:		Total	Gain per Pig	Therms per lb Gain	Weaning Weight per Pig
			Sows' Milk	Creep				
Summer:								
6 + 1	5	33	49.4	21.7	71.1	35.6	2.00	40.5
4 + 3/4	5	30	49.0	26.8	75.8	35.7	2.12	40.7
Autumn:								
4 + 3/4	4	34	48.4	19.2	67.6	33.2	2.04	38.5
3 + 1/2	5	37	51.5	20.5	72.0	33.9	2.12	39.0

There is no apparent relationship between sow milk energy intake and creep energy intake in these data. There is also no effect of treatment upon final weaning weight, all groups being satisfactory in this respect.

The final comparisons between treatments are those based on the energy recovered in the litters as body tissue. In Table 7 are shown:

1. The gross efficiency of milk production, i.e. percentage recovery of sow feed energy in the form of milk energy.
2. The percentage of sow milk and creep feed energy recovered as litter energy.
3. The percentage of sow feed and creep feed energy recovered in the litters, and
4. The percentage of sow feed energy alone, recovered in the litters, the energy content of weight gains due to creep intake having been excluded.

In each case the sow feed energy has been adjusted by adding or subtracting the energy equivalent of sow weight changes.

TABLE 7. COMPARISONS OF PRODUCTION EFFICIENCIES

Seasons	Summer		Autumn	
Treatments	6 + 1	4 + $\frac{3}{8}$	4 + $\frac{2}{8}$	3 + $\frac{1}{2}$
Gross efficiency of milk production	39.9%	47.9%	48.2%	52.7%
% of sow milk and creep energy recovered in litters	54.7%	51.7%	54.1%	51.6%
% of sow feed and creep energy recovered in litters	25.3%	29.8%	29.4%	30.3%
% of sow feed energy recovered in litters	20.4%	24.2%	24.9%	26.0%

Table 7 emphasises the inefficiency of the double processing which occurs when feed is converted to milk by the sow, and this milk is converted by the litter into tissue energy. Whereas more than 50% of the metabolisable energy contained in sow's milk and creep feed was recovered in the litter, only 20% to 26% of the metabolisable energy fed to the sow was finally retained as tissue energy by the litter. Table 7 also indicates that the litters on the sows fed the lower rations recovered a greater percentage of the sow feed energy in their weight gains than did the litters of sows on the 6 + 1 ration.

On the basis of the data shown here, sow feed energy was converted to litter energy 30% more efficiently when the sows were fed on the 3 + $\frac{1}{2}$ ration level than when fed on the 6 + 1 level.

GENERAL DISCUSSION

In the original study on sow milk production in this series, it was noted that the yields of the sows used were in excess of those found

by previous workers. It was suggested that this might be due to the inclusion of separated milk in the ration (Smith 1952). More recent studies (Smith 1959) show that while a ration of separated milk plus concentrate, or milk only, supported a higher milk yield than concentrate alone, the level of production achieved was below that of the sows in the original experiments. There was, however, a difference in ration levels between the two series, the original sows having received a $6 + 1$ ration while the milk plus concentrate experiments were conducted at the $4 + \frac{2}{3}$ level. Two possible explanations were then suggested for the lower yields in the second study, viz:

1. That the original sows were inherently superior producers, and
2. That the lower rations had depressed production.

This latter hypothesis may now be discarded, and it appears most likely that the high yields recorded in the original study arose from the use of inherently superior milk-producing sows.

The daily yields recorded in the experiments reported here, 10 to 13.6 lb, are of the same order as those reported by Wells, Beeson, and Brady (1939), 11.7 lb, Berge and Indrebo (1953), 13.6 lb, and Niwa *et al.* (1951), 9.3 lb. The yields of the sows used in the Ruakura experiments comparing milk and meal varied between 10.8 and 13.3 lb per day (Smith 1959).

Three other aspects of these experiments merit further discussion. The first is the ability of the sow to buffer her litter against the effects of a quantitatively poor ration by the use of body reserves. Any person concerned with extension work in pig husbandry will at some time have been disconcerted by the fact that poorly fed sows in some cases appear to wean as heavy and thrifty pigs as do better-fed ones. To some extent this fact does appear to discount the advice usually given, that sows require a reasonably high level of feeding in order to produce sufficient milk to nourish their young adequately.

On a short-term basis this advice is incorrect, and the present study offers a reasonable explanation for the apparent anomaly. However, it is unlikely that a sow which becomes severely depleted while using her body reserves to support a high level of milk production in the course of one lactation, could repeat the process in the next, unless she were given the opportunity to recover the weight lost. In addition, sows that wean in poor condition are more often than not difficult to remate successfully at the first or second heat after weaning, and thus annual production is reduced in proportion to the time required by the sow to recover sufficient condition to breed again (Ballinger 1944). The extent to which the sow must be reduced in condition before milk production is affected will be the subject of future studies at this Station.

It would appear that the use of body reserves which are, initially at least, mainly fat depots, results in an increased fat content in the milk produced. Willet and Maruzama (1946) have shown that an increase in fat intake by the sow results in an increased milk fat content, and it appears likely that the utilisation of body fat by the sow has a similar result in terms of the milk constituents. Similarly, Brody

(1945) reported that in the rather extreme case of fasting dairy cows, the fat percentage of the milk rose and the lactose content fell. In the reported experiments, however, the total milk and fat yield also fell.

The second aspect for discussion is the increased efficiency of milk production as the ration level was lowered. Armsby and Moulton (1925) and Forbes *et al.* (1926) have shown that in dairy cows the average efficiency of utilisation of the net energy of the ration for maintenance, lactation, and weight gains was in the ratio of 1 for maintenance, 0.985 for lactation, and 0.761 for body increase.

Since the present data do not involve weight gains, the significance of these findings relates only to maintenance and milk production, for which purposes the animal apparently uses its feed with similar efficiency.

Later work by Forbes *et al.* (1928) on the energy metabolism of steers indicated that the efficiency of feed energy utilisation decreased when the intake was raised from half-maintenance to a point of energy equilibrium. If the previous finding that feed energy is utilised as efficiently for milk production as for maintenance holds true for a sub-maintenance feeding regime, then the increased efficiency of milk production on the $\frac{2}{3}$ and $\frac{1}{2}$ feeding levels in the present experiments finds a ready explanation.

Since the increased efficiency of milk production was correlated with increased weight losses, another possible explanation for the findings is that the estimated "energy intake equivalent" of a pound loss in weight, 3312 kilocalories, is too small. If this were so, the sows losing most weight would show to advantage.

It is unlikely that the actual energy content of the tissues involved in weight losses has been underestimated, since the values were based upon direct determinations (Pomeroy 1941; Hankins and Ellis 1945). It is more likely that the energy content was overestimated, especially over the latter part of lactation when the severely depleted sows could well have been deriving energy from the catabolism of protein rather than fat.

If it is accepted that the energy content assigned to the tissues is correct, the question arises whether during catabolism the energy derived from body depots is strictly comparable to the metabolisable energy of the ration. There are, unfortunately, no reliable data upon the losses involved in the catabolism of body tissue at submaintenance levels of nutrition, and, therefore, it is impossible to compare these losses with those which take place in the process of converting the metabolisable energy of the ration into net energy.

It does appear from the data on weight losses and efficiency within groups (Table 4) that there is little or no correlation between the magnitude of weight loss and efficiency of milk production of individual sows, and the same conclusion may be drawn from the data presented in the previous study in this series (Smith 1959). It is probable, therefore, that no large errors are involved in accepting the energy content of body reserves as being equivalent in nature and availability to the metabolisable energy of feed intake.

The third aspect of these experiments which merits comments is the efficiency of lactation as shown by the percentage of sow energy intake equivalent that is finally recovered in the litter. Brody (1945) states that about 30% of the t.d.n. intake of above-average dairy cows is recovered in the energy of the milk produced. From the present data a reasonable value for the efficiency of milk production of sows would be 45%. Even when the milk energy is further processed by the suckling pig, the final percentage recovery in piglet tissue is between 20% and 26%, which must be regarded as a relatively high standard of biological efficiency. The inclusion of creep energy intake and the additional litter gains derived from this supplementary feed raised the efficiency values by approximately 5% which, once again, emphasises the importance of creep feeding in the economy of pig keeping, and also focuses attention upon the theoretical advantages of early weaning as a technique for further improving the energetic efficiency of the lactational phase of the reproductive cycle.

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SPORE PRODUCTION BY *Sporidesmium bakeri* ON RYECORN (*Secale cereale*)

By A. B. LLOYD, Seed Testing Station, Department of Agriculture, Palmerston North,* and R. T. J. CLARKE, Plant Chemistry Division, Department of Scientific and Industrial Research, Palmerston North

(Received for publication, 29 July 1959)

The growing of *Sporidesmium bakeri* on ryecorn (*Secale cereale*) is described. The method is simple and large quantities of clean, toxic spores are easily obtained.

In 1959 Thornton and Percival reported that felts of *Sporidesmium bakeri*, grown on potato-carrot agar, and containing hyphae, spores, and medium, produced liver damage in guinea pigs indistinguishable from that obtained by feeding hepatotoxic grass. Similar material produced liver damage characteristic of facial eczema in lambs. It has since been shown that a considerable proportion of the toxic material is present in the spores (Thornton, pers. comm.). With these discoveries there arose the need for a method of obtaining large quantities of clean, toxic spores of the fungus. Spores are produced in large numbers by *S. bakeri*, strain C, on media such as potato-carrot agar, but it is difficult to recover the spores dry or relatively free from fragments of hyphae, agar, and other debris. Various natural media and substrates were tested in an endeavour to find a simple method of obtaining a satisfactory yield of spores. The best of these was autoclaved seeds of ryecorn (*Secale cereale*).

Ryecorn seeds were soaked overnight in tap water, dispensed without free water in 4 in. layers in quart milk bottles, and autoclaved at 10 lb for 30 min. After inoculation with spores the bottles were incubated at 23–25°C for 12–16 days.

The spores were easily recovered dry or in aqueous suspension. When the bottles were shaken, a dense cloud of spores arose which was easily removed by suction. Shaking with water gave a similar recovery of spores which could be separated from the seed material by filtration through gauze. The spores obtained were relatively free from hyphae and pieces of the substrate, which is difficult to achieve when the spores are recovered from agar media, autoclaved peas, and similar material. The spores obtained were highly toxic to guinea pigs.

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FREYBERG, A NEW APPLE VARIETY

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(Received for publication, 26 August 1959)

Summary

Freyberg, a new apple variety of Golden Delicious $\times$ Cox's Orange Pippin parentage is described. It is a yellow apple of very good flavour maturing at the same season as Golden Delicious which it most resembles. It is recommended for trial as a high-quality desert apple for home gardens.

Though a number of apple varieties of local origin have been important in the New Zealand fruitgrowing industry in the past, the only one of importance in today's commercial apple orchards is Kidd's Orange Red, a cross between Cox's Orange Pippin and Delicious, raised in 1912 by J. H. Kidd of Greytown. The good qualities of this apple stimulated Mr. Kidd to make further crosses, and at the time of his death he had raised a number of young seedlings of various parentages. These trees were given by Mr. Kidd's executors to the Department of Scientific and Industrial Research. To conserve space, scions from the seedlings were worked on to the dwarfing Malling IX rootstock and the resulting trees were planted, some at the Research Station at Mt. Albert, Auckland, and the remainder at the Appleby Research Orchard, Nelson.

When these trees commenced bearing they were kept under observation for a number of years and one seedling, N 21, of Golden Delicious $\times$ Cox's Orange Pippin parentage, was selected as a promising type. It has now been decided to name this selection and distribute it for trial.

The apple has been named Freyberg out of respect for Lord Freyberg, commander of the 2nd New Zealand Expeditionary Force in World War II, and Governor-General of New Zealand 1946 to 1952, who graciously permitted his name to be used.

It is a medium-sized, yellow apple maturing about the same season as Golden Delicious which it most resembles. It is rather less attractive in appearance than Golden Delicious but the flavour, which combines some of the good qualities of both parents, is preferred by many. The aromatic aniseed flavour is influenced by seasonal conditions and is occasionally rather too pronounced for those preferring American varieties.

Because of its superior eating quality, and regular, heavy-bearing habit, Freyberg would be a first-rate variety for the home garden, and is worth limited trial for commercial growing. Since the open passage from calyx tube to core renders the apple liable to mouldy core, Freyberg is not recommended for commercial planting in the Auckland district, and since it would compete directly on the market with Golden Delicious it should be tested only on a restricted scale in other commercial fruitgrowing areas.

Fruit Description

The following description applies to fruit from dwarf trees on Malling IX stock grown at Mt. Albert, Auckland. A planting of standard-sized trees of Freyberg on Merton 793 is still too young to permit an accurate tree description being made.

Size. Medium, diam. $2\frac{7}{8}$ in., height $2\frac{3}{8}$ in. fairly uniform.

Shape. Roundish, varying to roundish conic, slightly crowned, sides often a little unequal.

Basin. Medium in width, medium to deep, slightly creased, slight, smooth russeting in patches. Calyx closed to slightly open, lobes medium size, long, acuminate, reflexed at tips.

Cavity. Narrow, medium to deep, stalk medium in length and thickness.

Skin colour. Greenish yellow to cream, turning golden yellow when mature, about 5% of the fruit having a thin wash of colour on $\frac{1}{3}$ of the surface, pale carmine becoming dull orange when the fruit is mature.

Lenticels. Numerous, medium-sized, brown with yellowish-white halo, indistinct on ripe fruit.

Skin. Thin with very slight bloom.

Flesh. Fine-grained, pale yellow, crisp, juicy, subacid, sweet and aromatic—slight aniseed flavour more pronounced in some seasons. Very good in quality.

Calyx tube. Funnel form, medium, long, sometimes open to core.

Core. Medium to small, slightly abaxile, cells open.

Seeds. Medium size, plump, chestnut brown.

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